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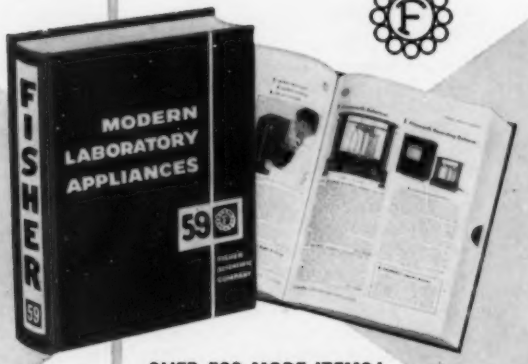

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***Peterson, E. T., Walford, R. L., Figueroa, W. G. and Chisholm, R. Ox Cell Hemolysins in Infectious Mononucleosis and in Other Diseases. Am. J. Med. *21(2):* 193-199 (Aug.) 1956. (Hyland Laboratories will send you a reprint of this article promptly, at your request.)**

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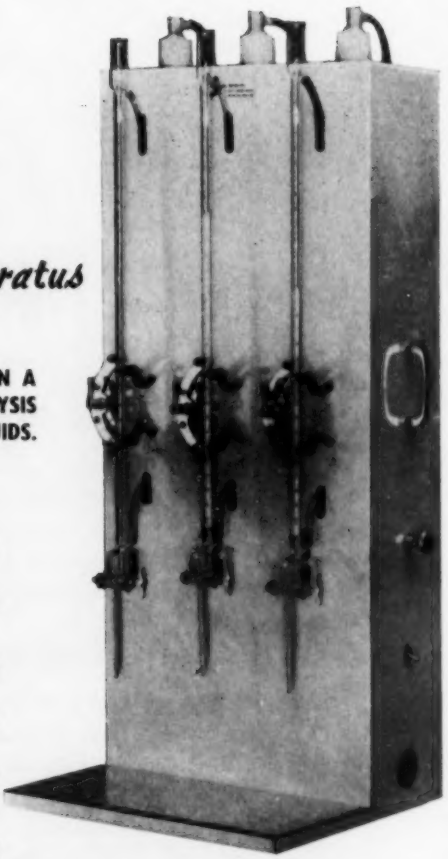

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1. Boland, E. W.: California Med. 88:417 (June) 1958. 2. Bunim, J. J., et al.: Arthr. & Rheum. 1:313 (Aug.) 1958 3. Boland, E. W., and Headley, N. E.: Paper read before the Am. Rheum. Assoc., June 21, 1958, San Francisco, Calif. 4. Bunim, J. J., et al.: Paper read before the Am. Rheum. Assoc., June 21, 1958, San Francisco, Calif.

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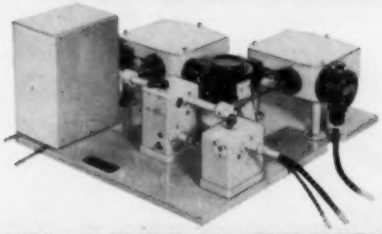

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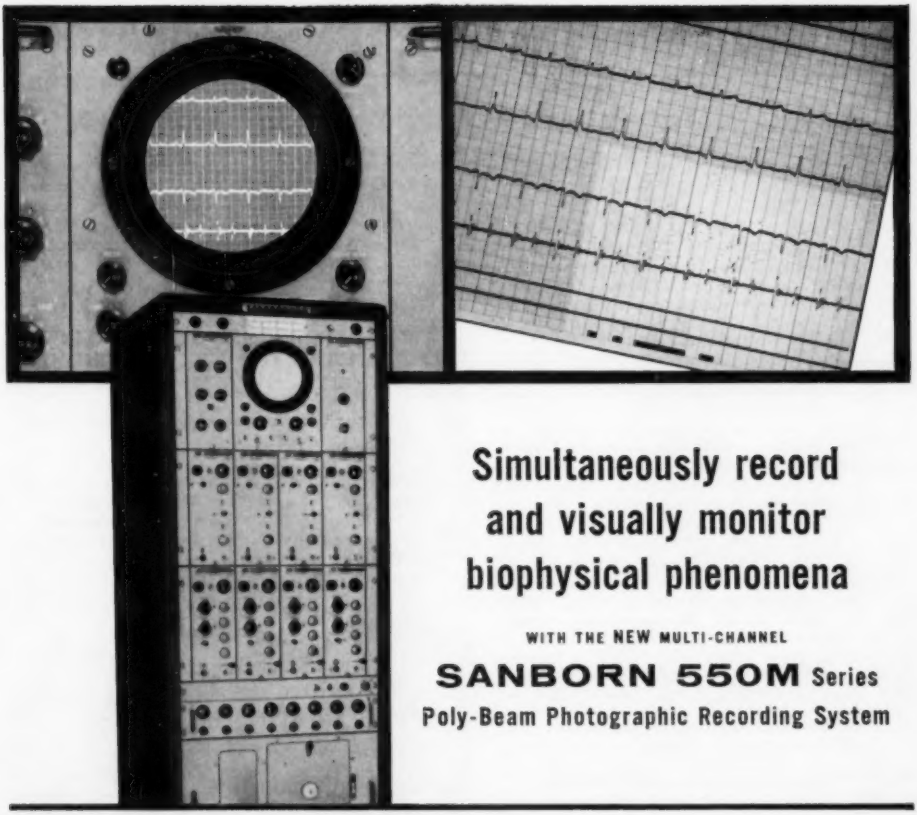

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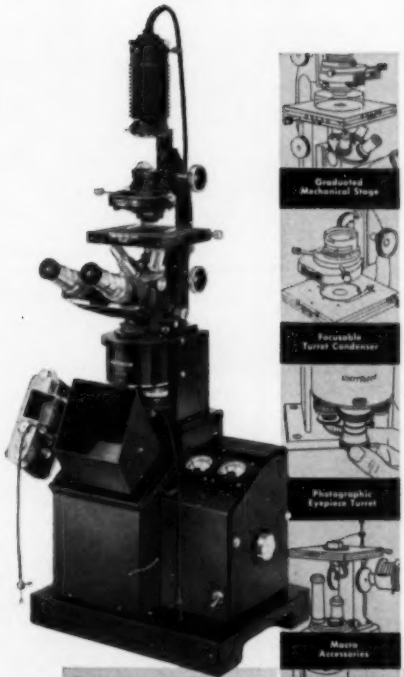

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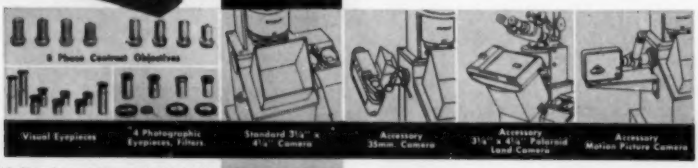
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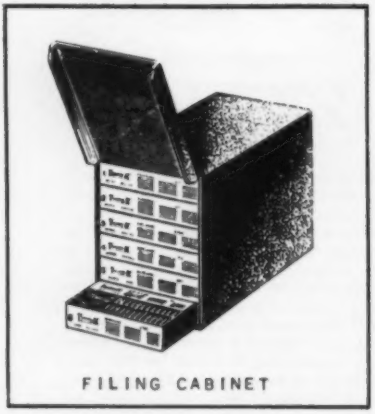

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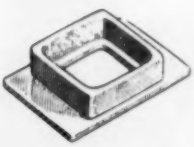

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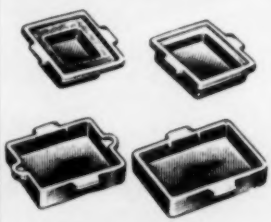

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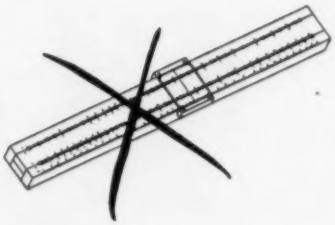

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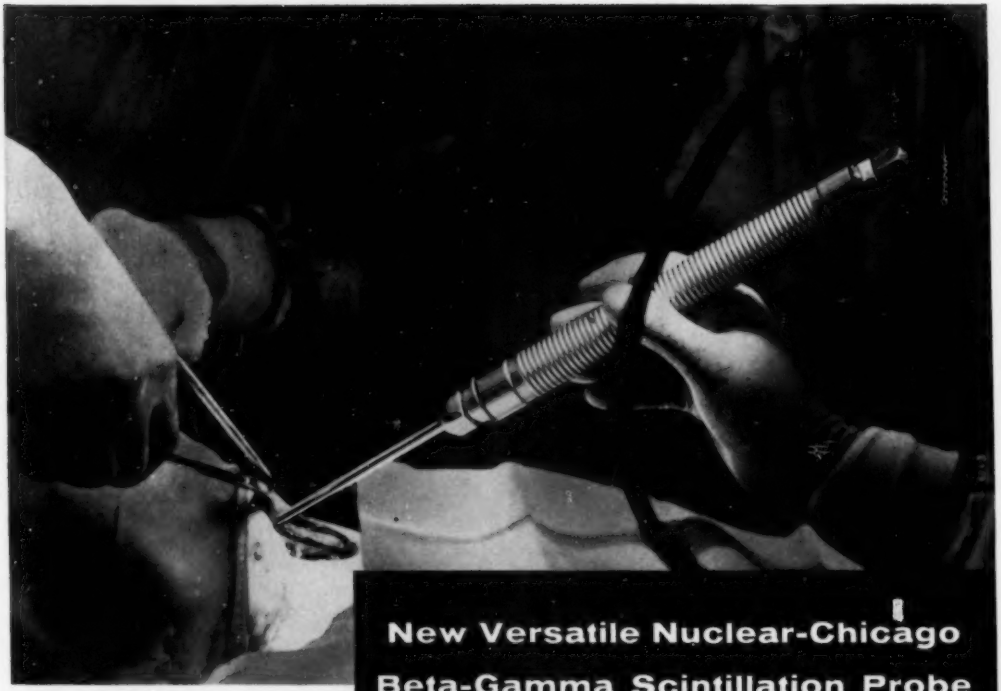

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CHARLES A. JACOBI, Head, X-Ray Technology, Oregon Technical Institute, Oretech, Oregon, and **DONALD E. HAGEN,** Instructor, X-Ray Technology, Oregon Technical Institute, Oretech, Oregon. With Foreword by **JAMES M. HILTON,** M.D., Roentgenologist and Course Supervisor, X-Ray Technology, Oregon Technical Institute.

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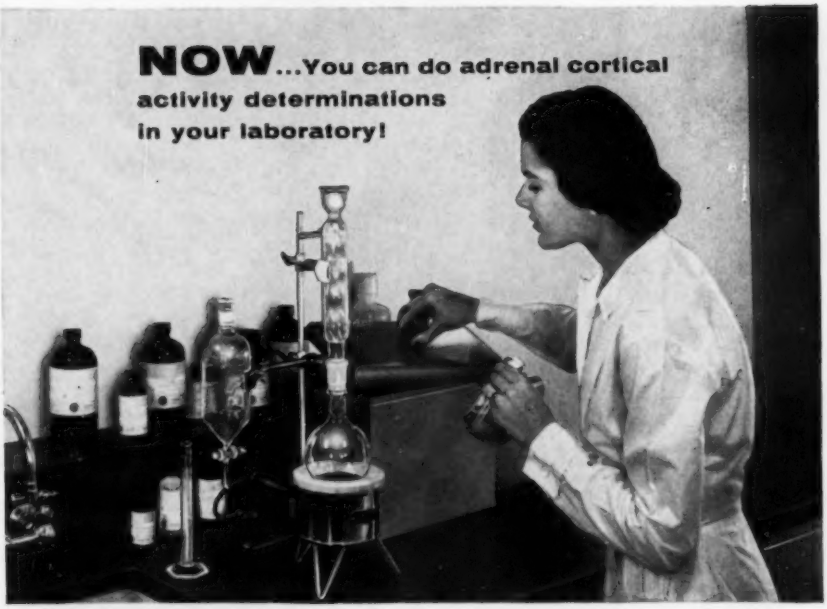

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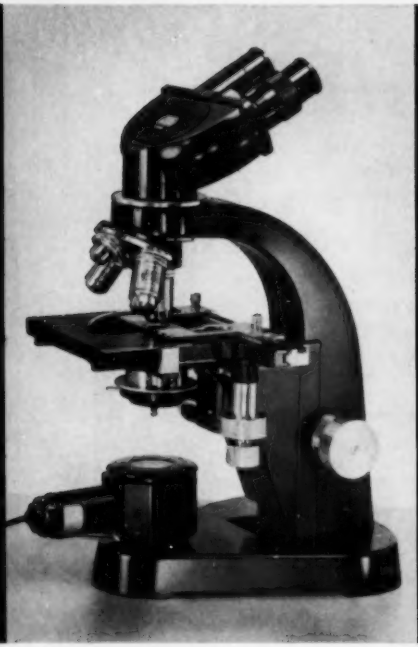

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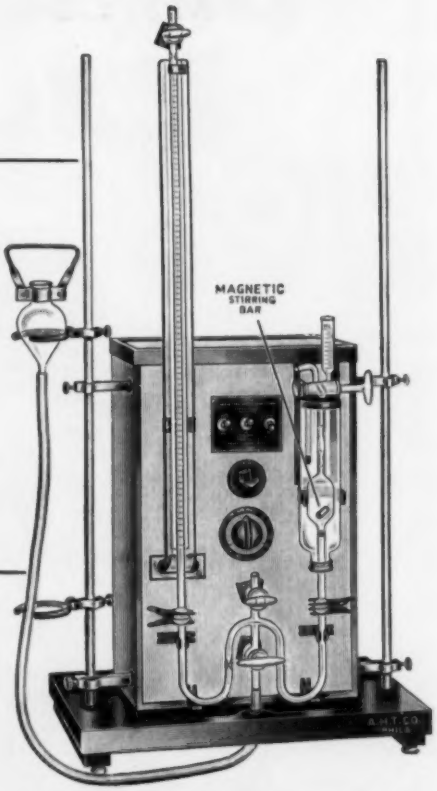

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The Journal of Laboratory and Clinical Medicine

VOL. 52 DECEMBER, 1958 No. 6

CLINICAL AND EXPERIMENTAL

LIMITATION OF RADIOIODINE AS A LABEL FOR FAT

E. VAN HANDEL, PH.D., AND D. B. ZILVERSMIT, PH.D.
MEMPHIS, TENN.

FATS iodinated with I^{131} have been used for the measurement of fat absorption in man. In malabsorptive disorders, both the total amount of I^{131} and the lipid-bound I^{131} appearing in blood are significantly depressed.[1-3] Although fat balance studies, fecal fat determinations, and chylomicron counting have provided useful information, the use of tagged fats offers obvious advantages over the classic procedures. The use of C^{14} fat in man is limited, however, by the expense, the hazard of administering a long-life isotope, and the difficult assay of the soft beta rays of C^{14}. Radioiodine eliminates some of these objections and if it could be shown that iodinated fat quantitatively behaves like a normal fat during absorption and deposition it would be a powerful tool for the study of fat metabolism in man and experimental animals.

In this study the validity of I^{131} labeling has been tested by: (a) comparing I^{131} lipid radioactivity with chemically determined fat in lymph and lipemic blood, (b) mixing I^{131}-labeled fat or fatty acid with C^{14}-labeled fat or fatty acid and determining the ratio lipid I^{131}-C^{14} in lymph fat and in the neutral fat and phospholipid fractions of tissues.

MATERIALS AND METHODS

For the study of fat disappearance mongrel dogs were injected with a fat emulsion which was prepared by mixing I^{131} fat* with carrier fat and the dropwise addition of this

From the Department of Physiology, University of Tennessee, Memphis, Tenn.
This investigation was supported by Research Grant H-2181 from the National Heart Institute, Public Health Service.
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*Triolein I^{131}; Abbott Laboratories, Chicago, Ill.

fat to a glycerol phosphatide base[4] in a Waring blendor. Aliquots of this anhydrous base were then mixed with 5 per cent dextrose to give an emulsion of the following composition: fat, 20 per cent; phosphatide, 2 per cent; dextrose, 3 per cent; and glycerol, 20 per cent. About 10 ml. of the emulsion containing about 2 Gm. of cottonseed or coconut oil was administered intravenously to dogs anesthetized with sodium pentobarbital. Blood samples were removed and whole blood was subjected to the chemical procedures described below. In the experiments with intravenous emulsions, whole blood was used rather than plasma since during centrifugation fat accumulates at the top of the centrifuge tube, and this makes it difficult to obtain a representative aliquot for analysis.

For the study of lipemia, postabsorptive mongrel dogs were fed different kinds of fats to which was added a small amount of I^{131} fat. A total of 5 Gm. of fat per kilogram body weight was administered by stomach tube. Blood samples were obtained 2.5, 4, 5, and 6 hours after fat feeding.

In the experiments where dog lymph was collected, the thoracic duct was cannulated under sodium pentobarbital anesthesia. Sixteen hours later the dogs received a dose of labeled fat via a permanent polyethylene tube inserted in the esophagus. Lymph flow of about 0.5 ml. per minute was maintained by administration of water by esophageal tube. All animals were kept in restraining cages during the experiment.

Rats were fed labeled fats or fatty acids by stomach tube and sacrified 6 hours later. The carcass, exclusive of gastrointestinal tract and liver, was ground and an aliquot taken for lipid extraction. Several other rats were anesthetized with sodium pentobarbital, their cisterna chyli cannulated with polyethylene tubing, and lymph was collected while the animal was kept in a restraining cage. Saline was given intragastrically by a constant infusion pump at the rate of 1 ml. per hour. Labeled fats were also administered through the gastric cannula.

A. *Trichloroacetic Acid Precipitation*[1, 5].—Five milliliters of 10 per cent aqueous potassium iodide (KI) and 2 ml. of 40 per cent trichloroacetic acid (TCA) were added slowly to 1 ml. of whole blood or plasma. After centrifugation the clear supernatant was poured off and the precipitate stirred with an additional 5 ml. KI and 2 ml. TCA. The mixture was recentrifuged, decanted, and the precipitate counted in a well-type scintillation crystal. This fraction contains all lipid I^{131} plus any protein-bound I^{131} that might be present.

B. *Chloroform Methanol Extract.*—One milliliter of plasma was placed in a 25 ml. volumetric flask and made to volume with 2:1 chloroform-methanol. After mixing, the protein was removed by filtration. The tissues were ground with sand and methanol and then extracted with sufficient chloroform to obtain a chloroform-methanol solvent ratio of 2:1. Twenty milliliter aliquots were placed in glass-stoppered centrifuge tubes. Methanol and nonlipid I^{131} were removed by a modified Folch procedure[6] as follows: 20 ml. of water was added and the tube was carefully inverted several times and centrifuged at low speed until separation was complete. The aqueous supernatant was drawn off and discarded. The remaining chloroform was washed once again with 6 ml. of a water-methanol mixture (5:4) which had been saturated with a few drops of chloroform until just turbid. The residual methanol which would interfere with the chromatography was removed by washing the chloroform with an additional 6 ml. of water. An aliquot was counted for lipid I^{131} and the rest was poured through a fast-flowing silicic acid column.[7] Elution of nonphosphatide lipids was completed by adding chloroform to the column to make the total eluate equal to 25 ml. Subsequently phospholipids were removed with 25 ml. methanol.

C. *Zeolite Supernatant.*—One milliliter of whole blood, plasma, or lymph was mixed with 4 Gm. of zeolite* previously wetted with 2 ml. chloroform. After thorough mixing,

*Zeolite (formerly called Doucil) was obtained from W. A. Taylor Company, 7300 York Road, Baltimore, Md.

an additional 18 ml. chloroform was added, and the solution was shaken vigorously for 5 minutes. After standing for about one hour and a final shaking, the zeolite was filtered off and aliquots of the filtrate which contains I^{131} triglycerides plus whatever I^{131} cholesterol ester might be present were counted as before and used for the determination of total triglycerides.[7] C^{14} lipid was determined by a thin-window gas flow counter after mounting a small aliquot of chloroform extract in a stainless steel cup. In those experiments where I^{131} and C^{14} lipid were present in the same extract, the steel cups were also counted with an aluminum foil covering which absorbed 60 per cent of the I^{131} activity and 99 per cent of the C^{14} activity. In general the I^{131} determined in this manner was in good agreement with that obtained by gamma counting.

TABLE I. RECOVERY OF I^{131} FAT AND REMOVAL OF NaI^{131} BY DIFFERENT PROCEDURES

METHOD	EXPERIMENT	PER CENT OF ADDED I^{131} TRIOLEIN EMULSION*	PER CENT OF ADDED NaI^{131}
TCA precipitate	1	92.0	1.90
	2	91.5	1.90
Purified chloroform methanol extract	1	91.5	2.45
	2	92.5	2.47
Zeolite supernatant	1	92.5	0.67
	2	92.5	0.65

*This emulsion was stored at room temperature for several weeks and contained 8 per cent dialyzable I^{131}.

RESULTS AND DISCUSSION

Tests of Chemical Procedures.—For the determination of total lipid I^{131} the trichloroacetic acid precipitation is very convenient. However, this procedure does not furnish a sample which may be subsequently fractionated into the various lipids. For that purpose the extraction with chloroform-methanol is more suitable. To test the ability of these methods to recover added fat, 2 samples of plasma were divided into 3 aliquots each. To each aliquot was added a small amount of artificial fat emulsion containing I^{131} fat. Each procedure achieved virtually complete recovery of the added I^{131} lipid, whereas a maximum of only 2.5 per cent of I^{131} from NaI^{131} added to nonradioactive plasma was found as a contaminant in the lipid fraction (Table I). Procedures A, B, and C, under Materials and Methods, do not provide exactly the same type of information. Trichloroacetic acid precipitates all the plasma lipids and also protein-bound I^{131} if this is present. Purified chloroform-methanol also contains all lipids but is virtually free of I^{131} thyroxin. Zeolite supernatant, on the other hand, provides a thyroxin-free lipid extract containing no phosphatides. Such an extract may also be obtained by fractionation of the total lipid extract through silicic acid columns with chloroform.[7] That practically none of the I^{131} lipid represents cholesterol ester activity was demonstrated as follows: the chloroform eluate was evaporated by gently heating and the residue picked up in petroleum ether. The extract was applied to a column containing 2.5 Gm. of a silicic acid: Super-Cel (1:1) mixture and cholesterol ester was eluted with 25 per cent chloroform in petroleum ether (b.p. 60° to 80° C.). The cholesterol ester isolated from several lipemic

dog plasma samples contained less than 2 per cent of the I^{131} in the chloroform eluate. In other words, 98 per cent of the I^{131} was contained in the form of glycerides, presumably triglycerides. To demonstrate that iodinated cholesterol esters are eluted by 25 per cent chloroform in petroleum ether as are the naturally occurring cholesterol esters[8] we prepared I^{131}-labeled cholesterol ester by treating cholesterol oleate with I^{131} Hanus solution. Since cholesterol itself did not become labeled under our conditions it may be assumed that the I^{131} label was present in the fatty acid chain. Practically all the ester thus prepared could be recovered from the column with the chloroform petroleum ether mixture, and the material could be rechromatographed without change in specific activity.

Very little I^{131} lipid activity was eluted from the column by methanol and even that amount did not represent phospholipid activity. This was shown by addition of carrier phosphatides and repeated precipitation with acetone of total plasma lipid fractions which reduced the activity in the precipitate to less than 1 per cent of the total activity.

Disappearance of Intravenous I^{131}-Labeled Fat Emulsions.—In order to test the physiologic behavior of fat particles labeled with I^{131} triolein, the disappearance of lipid I^{131} administered as an artificial fat emulsion was compared to the disappearance of chemically determined fat. Before injection of the emulsion the whole blood triglyceride concentration was measured. At various intervals after injection of the emulsions whole blood triglycerides and lipid radioactivity were determined in the zeolite chloroform supernatant. The data in Table II show the percentage of the injected oil present per total blood volume at any one time after the injection. In each instance the removal of I^{131} triolein appears to proceed parallel to the removal of the total fat irrespective of the great differences in the fatty acid composition of the carrier fat used in making the emulsion. I^{131} triolein, therefore, furnishes a quantitative index for the removal of emulsified fat.

TABLE II. COMPARISON OF CHEMICAL AND RADIOACTIVE TRIGLYCERIDE AFTER INTRAVENOUS ADMINISTRATION OF FAT EMULSIONS

COTTONSEED OIL			COCONUT OIL			HYDROGENATED COCONUT OIL		
MINUTES	TRIGLYCERIDE	LIPID I^{131}	MINUTES	TRIGLYCERIDE	LIPID I^{131}	MINUTES	TRIGLYCERIDE	LIPID I^{131}
6	72	71	2	98	95	1.5	89	83
17	48	48	5	71	77	7.5	74	65
32	28	28	10	32	32	15	47	45
60	21	20	21	15	16	31	36	36
120	5	7	35	11	11	65	19	18
						140	11	14

The numbers represent the per cent of the injected chemical triglyceride or I^{131} lipid present per whole blood volume assuming 80 ml. of blood per kilogram body weight. Chemical triglycerides are corrected for zero time concentration.

Lipemia Tolerance Curves With I^{131}-Labeled Fats in Dogs.—Since most of the clinical studies have been carried out with orally administered I^{131} triolein it was of interest to test the behavior of this fat after oral administration. Thirteen fasting dogs received I^{131} triolein mixed with hydrogenated

cottonseed oil, hydrogenated coconut oil, lard, or triolein at a dosage of 5 Gm. per kilogram of body weight. At regular intervals lipid radioactivity and triglycerides were determined. A typical example is shown in Table III.

TABLE III. COMPARISON OF CHEMICAL AND RADIOACTIVE PLASMA TRIGLYCERIDE AFTER ORAL ADMINISTRATION OF TRIOLEIN TO A DOG

HOURS	TRIGLYCERIDE		PER CENT OF I^{131} TRIOLEIN PER LITER
	MG. PER CENT	PER CENT OF DOSE PER LITER	
0	41	—	—
2.5	244	4.8	3.4
4	194	3.6	2.5
5	214	4.0	3.4
6	164	3.0	2.8

Some dogs responded to the oral fat with appreciable hypertriglyceremia and lipid radioactivity. Other dogs failed to show appreciable turbidity and hypertriglyceremia and similarly had only little I^{131} lipid activity in their blood stream. In a semiquantitative manner, then, I^{131} triolein gave the same results as did the chemical determination of plasma triglycerides. However, in all instances where the triglyceride rose far enough above the fasting level so that chemical determination of that difference could be made with great accuracy, the increase in triglycerides was greater than the amount of lipid radioactivity; in other words, the specific activity of the excess plasma triglyceride was lower than that of the administered dose. This held true, no matter which one of the four types of fat were used to produce the hyperlipemia.

In view of the great similarity in clearing the I^{131} triolein and the carrier fats of the artificial emulsion it is unlikely that I^{131} was removed from the circulation at a rate different from that of the carrier fat. Therefore, it seemed more likely that the lowering of the specific activity of the fat fed occurred during the absorptive process. In order to study this discrepancy, the specific activity of the absorbed fat was studied before entering the bloodstream by collecting fatty lymph from dogs and rats.

TABLE IV. FAT CONTENT AND SPECIFIC ACTIVITY OF THORACIC DUCT LYMPH OF A DOG FED I^{131} TRIOLEIN

HOURS AFTER FAT FEEDING	TRIGLYCERIDE CONTENT (MG./ML.)	SPECIFIC ACTIVITY AS PER CENT OF FED FAT
before	2.1	
0-1	—	0
1-2.5	5.0	30
2.5-4	40.0	41
4-5	53.5	58
5-6	69.5	58
6-8	70.5	54

Lymph Fistula Animals.—The triglyceride content of thoracic duct lymph at various time intervals after feeding a dog 5 Gm. per kilogram of body weight of I^{131} triolein diluted with nonradioactive triolein carrier is shown in Table IV. The initial lymph sample contained only 2.1 mg. triglyceride

per milliliter of lymph but 2 hours later the fat content had increased more than twenty fold. It is interesting to note that the triglyceride in lymph even during maximal rates of fat absorption never reached a specific activity exceeding 60 per cent of the specific activity of the fed triglyceride. In two additional animals the total amount of triglyceride recovered in lymph 24 hours after the fat feeding was determined to be 88 and 97 per cent, respectively. In these same animals only 20 and 56 per cent of the administered I^{131} fat was recovered in the lymph, or the average specific activity of the lymph triglyceride over this period was 25 and 64 per cent of the fat fed, respectively. In order to exclude any aftereffect of surgery or anesthesia a dog was fed 5 Gm. per kilogram of triolein mixed with I^{131} triolein and anesthetized 3.5 hours after feeding. Immediately a small sample of lymph was withdrawn from the thoracic duct. In this animal the lymph contained 46.4 mg. triglyceride per milliliter but the specific activity was only 54 per cent of the administered dose.

The discrepancy between the specific activity of the fat fed and the specific activity of the lymph may be due to any of three causes: (a) the I^{131} triolein is partly delabeled before or during absorption, (b) the I^{131} triolein is absorbed by a different route or at a rate different from the carrier fat in which it is dissolved, and (c) I^{131} triolein behaves like the carrier fat, but the administered fat is diluted during absorption with a large amount of triglycerides from a nondietary pool. If either a or b were true, I^{131} triolein would not be considered a valid label for the carrier fat. In this case, I^{131} triolein would be expected to yield results different from the more accepted type of fat labeling with C^{14}. In the third case, however, I^{131} triolein would be a more accurate parameter of dietary fat than a chemical determination, as the latter would measure both the dietary and the endogenous pool. Here, I^{131}-labeled fat would be expected to yield the same information as C^{14}-labeled fat.

In order to test the foregoing possibilities, lymph was collected from rats after oral feeding of 0.5 ml. of a mixture of C^{14} triolein and I^{131} triolein. Because of the scarcity of C^{14} triolein, rats were used after it was established that rat lymph showed a similar decrease in specific activity of fed I^{131} triolein as did dog lymph. The results are shown in Table V. In 3 rats the I^{131} lipid recovered from lymph amounted to 30 to 55 per cent of the C^{14} lipid. In the third rat triglycerides were also determined chemically, and in this rat the specific activity, measured as the ratio of C^{14} to triglycerides, is practically the same as for the administered fat. Therefore, it is obvious that dilution with endogenous lipid cannot account for the observed differences between I^{131} activity and chemically determined fat content of lymph. No matter whether part of the I^{131} is broken off the fat or whether I^{131} triolein is absorbed at a rate different from triolein, I^{131} does not appear to give a quantitative measure of the amount of fat absorbed. However, this finding does not necessarily affect the use of I^{131} triolein as an empiric clinical test for malabsorption syndromes.

TABLE V. I^{131} AND C^{14} TRIGLYCERIDES IN THORACIC DUCT LYMPH OF THREE RATS FED LABELED TRIOLEINS

HOURS AFTER FEEDING	LIPID I^{131}	LIPID C^{14}	LIPID I^{131} / LIPID C^{14}
3-5	1.8	3.5	.51
5-9	1.1	2.0	.55
9-20	0.37	0.94	.39
3-6	1.1	2.5	.44
6-9	0.55	1.1	.50
			Chemical Triglyceride
2-4	1.2	3.1	3.6
4-6	1.0	3.1	2.7
6-8	1.9	4.7	4.9
8-12	1.2	2.8	3.1
12-24	1.0	2.0	2.4

I^{131}, C^{14}, and chemical triglycerides are expressed as a per cent of the administered dose per milliliter of lymph.

The Incorporation of I^{131} Oleic Acid Into Tissue Lipids.—Investigators who have used C^{14} fatty acids have emphasized the extensive incorporation of these acids into liver phosphatides.[9-11] Artom,[12] on the other hand, some 25 years ago, used iodinated fat of high iodine content and found rather small amounts of iodine in several phospholipid fractions. A study of the labeling of tissue phospholipids would, therefore, yield yet another criterion for the validity of I^{131}-labeled fats in metabolic experiments.

At the time of these experiments C^{14} triolein, used in the lymph studies, was not available and, therefore, we compared I^{131} oleic acid* and C^{14}-labeled fatty acids. Since it has been shown that incorporation of fatty acids into tissue lipids depends on chain length and degree of unsaturation,[10, 11] and since the introduction of halogen into oleic acid might alter the physiologic behavior of oleic acid, we compared the incorporation of I^{131} oleic acid into liver and carcass phospholipids with both C^{14} oleic and C^{14} stearic acid. Table VI shows the results of these experiments. It is obvious that the I^{131} activity in the liver phosphatides is much smaller than either the oleic acid or stearic acid C^{14} activity. The methanol fraction from the silicic acid column even contained some impurities and when acetone precipitation was used to purify the liver phospholipids the phosphatide C^{14} activity after oleate or stearate feeding exceeded the I^{131} activity more than one hundred fold.

The distribution of I^{131} and C^{14} fatty acids in the triglyceride fractions of carcass agree more closely than might be predicted from the triolein feeding experiments where gross discrepancies between C^{14} and I^{131} in lymph were demonstrated. This suggested that appearance of I^{131} oleic acid in lymph after fatty acid feeding was more closely related to the appearance of C^{14} fatty acids than was the case with triolein. To test this possibility we collected lymph from 2 rats after oral administration of a mixture of C^{14} stearic and I^{131} oleic acids. Table VII shows that the ratio of I^{131} and C^{14} in the lymph triglyceride fraction (zeolite supernatant) was close to unity.

*Oleic acid I^{131}; Abbott Laboratories, Chicago, Ill.

Although from these experiments we cannot conclude that the absorption of I^{131} oleic acid differs from that of C^{14} stearic acid the lack of incorporation of the iodized fatty acid into liver phosphatides limits the use of I^{131}-labeled oleic acid in metabolic experiments.

TABLE VI. LIPIDS AS PER CENT OF THE FED DOSE PER WHOLE LIVER OR CARCASS SIX HOURS AFTER FAT FEEDING

RAT	ORGAN	METHANOL ELUATE*		CHLOROFORM EULATE†	
		I^{131}	C^{14}	I^{131}	C^{14}
1	Liver	0.03	8.5	0.50	1.2
2	Liver	0.03	1.74	0.17	0.18
	Carcass‡	0.52	4.6	10.7	11.6
3	Liver	0.009	0.068	0.089	0.162
	Carcass‡	0.93	1.30	28.6	28.8
4	Liver	0.024	0.59	0.142	0.51
	Carcass‡	0.16	0.29	6.5	8.2

Rats 1 and 2 received I^{131} oleic acid and C^{14} stearic acid dissolved in triolein.
Rats 3 and 4 received I^{131} oleic acid and C^{14} oleic acid dissolved in triolein.
*The methanol eluate contains primarily phosphatides.
†The chloroform eluate contains primarily triglycerides.
‡Gastrointestinal tract not included.

TABLE VII. I^{131} AND C^{14} TRIGLYCERIDES IN THORACIC DUCT LYMPH OF TWO RATS FED I^{131} OLEIC ACID AND C^{14} STEARIC ACID DISSOLVED IN TRIOLEIN

HOURS	I^{131}*	C^{14}*
2.5-5	2.8	2.5
5-8	1.8	1.8
1-5.5	5.8	5.2
5.5-7.5	2.3	2.3
7.5-24	1.7	1.9

*Per cent of administered dose per milliliter of lymph.

SUMMARY

Chemical procedures for the determination of I^{131} lipid fractions in whole blood, plasma, and tissues are discussed. I^{131}-labeled triolein, administered intravenously as an emulsion, disappears at the same rate as natural fats. After oral administration of I^{131} triolein the concentration of I^{131} triglyceride in dog plasma during the period of alimentary lipemia is lower than that of the chemically determined fat. This difference between the specific activity of fed fat and plasma fat already exists in lymph, as shown by the low specific activity of dog and rat lymph compared to that of the administered fat. Comparison of I^{131} and C^{14} triolein showed that dilution by an endogenous triglyceride pool cannot account for the observed decrease in specific activity. The distribution of orally administered C^{14} oleic acid and C^{14} stearic acid in the neutral fat fraction of carcass is fairly similar to that of I^{131} oleic acid. However, in liver, where rapid incorporation of C^{14}-labeled fatty acids into phospholipid fractions occurs, I^{131} oleic acid was found to be absent and thus gives an erroneous picture of the distribution of dietary fatty acids.

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EVALUATION OF A METHOD FOR DETECTING AND FOLLOWING URINARY TRACT INFECTION IN FEMALES WITHOUT CATHETERIZATION

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IT IS traditionally stated that urine specimens for evaluation of infections in females should be obtained by catheter, voided specimens being worthless because of contamination from the external genitalia.[1-3] On the other hand, despite the frequency and lack of hesitation with which it is performed, there is considerable evidence that bladder catheterization is not without danger of actually producing urinary tract infection by introduction of bacteria into the urinary tract.

It would seem that the only recourse for the physician is to balance the risk inherent in bladder catheterization against the possibility of overlooking urinary tract infection with its potential hazards.

It is generally appreciated that patients immobilized by poliomyelitis and other paralytic diseases are particularly prone to develop infections of the urinary tract. In order to detect and follow the course of urinary infections in a group of extensively paralyzed poliomyelitis patients without resort to catheterization, a method of obtaining satisfactory urine specimens for culture in female patients by a voided technique in conjunction with quantitative considerations was explored.

It is the purpose of this paper to report the results with this method and to discuss some of the problems in the diagnosis of urinary tract infection.

METHODS AND MATERIALS

Subjects for this study included 34 females with extensive paralytic poliomyelitis admitted to the Poliomyelitis Respiratory Center and 8 female patients of varying ages with no clinical findings indicative of urinary tract infection admitted to other hospital divisions. The poliomyelitis patients represented consecutive admissions to the Poliomyelitis Respiratory Center with the exception of a few patients who were hospitalized for only a brief period. Twenty-one of the poliomyelitis patients were over 15 years of age and 13 were younger. Twelve were admitted in the acute stage of poliomyelitis and 22 entered 30 or more days after the onset of poliomyelitis. All were extensively paralyzed, the extent of paralysis varying from 32 per cent to 94 per cent with a medium of 76.1

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per cent.* Virtually all of these patients were immobilized and recumbent for long periods. All except 2 experienced respiratory failure necessitating mechanical respiratory aids.

Urine for study was collected by the voided technique to be described at weekly intervals, or more often, in the poliomyelitis patients regardless of evidence of urinary tract infection. The first specimen passed by the patient each morning was discarded; the routine bath was then given with particular attention to cleansing the perineal area and the external genitalia. The second specimen was preferred rather than the first specimen on awakening to decrease the opportunity for multiplication of organisms in the bladder urine. Two teams, each consisting of a graduate nurse and two auxiliary nursing aids, collected all voided urine specimens from the patients during the study. With the patient in the dorsal lithotomy position, the external genitalia were scrubbed thoroughly with soap, followed by water and benzalkonium hydrochloride 1:1,000. The patient was encouraged to void and the specimen was collected from the midportion of the urinary stream in a sterile container and immediately transported to the laboratory. It was either processed at once or stored in ice for a time not exceeding 3 hours.

Bladder catheterization to obtain urine for comparison with the results of the voided specimen was carried out one or more times in the 34 poliomyelitis patients. In all instances the catheterized specimen was obtained on the same day as the corresponding voided specimen, usually between 6 and 8 hours later.

In order to compare the results of the techniques in nonparalyzed patients, paired specimens were collected by the two procedures from 8 women, all without evidence of urinary tract infection, admitted to general hospital divisions. Sixty-nine paired specimens were obtained from the poliomyelitis patients and 12 from the nonpoliomyelitis patients for a total of 81.

Each specimen was examined for pH, specific gravity, and presence of protein, reducing substance, and abnormal microscopic sediment. A gram-stained smear of an uncentrifuged portion of urine from each specimen was made. Bacterial strains were isolated by conventional methods and identified by standard cultural characteristics and biochemical fermentation reactions. Colony counts of the fresh, uncentrifuged urine were carried out by the method described by Knight and associates.[4] Drug susceptibility studies using the filter paper disk method were made on all positive cultures.

The details of other laboratory procedures and clinical evaluation of the poliomyelitis patients have been described in another publication.[5]

RESULTS

Comparison of Species Isolated and Colony Counts of Urine Specimens Obtained by Voided and Catheterization Methods.—In 42 patients the bacterial species isolated from specimens obtained by the voided technique were compared with those isolated from the corresponding catheter specimens. Table I depicts the species of organisms isolated by the respective techniques from consecutive weekly specimens in 8 representative female patients. In all patients the catheterized specimen was obtained on the same day as the corresponding voided specimen and, in most instances, between 6 and 8 hours later. The results show good correlation between the two methods in that the same bacterial species were usually isolated in paired specimens and cultures sterile by one procedure were also most often sterile by the other.

This type of qualitative comparison was carried out during the course of a long-term study in a large number of patients with good correlation between the results of the two techniques.[5]

*Based on Muscle Evaluation Standards as used by the National Foundation for Infantile Paralysis in the poliomyelitis vaccine trials.

TABLE I. COMPARISON OF BACTERIAL SPECIES ISOLATED FROM CLEAN VOIDED AND CATHETERIZED URINE SPECIMENS FROM EIGHT FEMALE PATIENTS. PAIRED SAMPLES OBTAINED ON SAME DAY*

PATIENT	TYPE SAMPLE	SAMPLE NUMBER 1	2	3	4	5	6	7	8	9	10	11	12
1	V	A	A,P	P		P	P		P,A	P,A	A	A	A
	C	A	A,P		P	P		P,A			A		
2	V	P	NG	P	P		P	P	NG	NG	ST		NG
	C			P		P			NG			NG	
3	V				P	P	P,A	P,A	P,SF	P,SF			
	C	A	P	P	P			P,A	P	P,SF			
4	V	A		A	A	A	A	A					
	C		A			A							
5	V	NG	A	A	A	A,P	A,P	A					
	C			A		A,P			A				
6	V			A	A	A		A					
	C	A	A				A						
7	V			P	P	NG	P	P					
	C	NG	NG		A	NG		P					
8	V	NG	NG	NG	NG	Co	Co	NG	NG	NG	NG	NG	NG
	C						Co	NG					

Key: A = *A. aerogenes;* P = Proteus species; ST = Staphylococcus; SF = *Str. faecalis;* Co = *Esch. coli;* NG = No growth; V = Clean voided urine sample; C= Catheterized urine sample.

*Blank areas indicate no sample was obtained.

TABLE II. COMPARISON OF BACTERIAL COLONY COUNTS OF CLEAN VOIDED AND CATHETERIZED URINE SPECIMENS

NUMBER PAIRED SPECIMEN	NUMBER BACTERIA PER MILLILITER IN CATHETERIZED SPECIMENS	NUMBER BACTERIA PER MILLILITER IN CORRESPONDING VOIDED SPECIMEN 0	$< 10^5$	$> 10^5$
28	0	17	9	2
25	$< 10^5$	0	23	2
28	$> 10^5$	0	0	28
81		17	32	32

TABLE III. COMPARISON OF NUMBER OF BACTERIA IN VOIDED AND CATHETERIZED URINE SPECIMENS FROM 42 FEMALE PATIENTS

DIFFERENCE BETWEEN SPECIMENS	NUMBER OF PAIRS
Both specimens sterile	17
Less than tenfold	46
Ten to one hundred fold	15
One hundred fold or more	3
Total	81

TABLE IV. COMPARISON OF NUMBER OF BACTERIA* IN PAIRED VOIDED AND CATHETERIZED URINE SPECIMENS IN FEMALE PATIENTS

AGE (YEARS)	DISEASES	VOIDED	CATHETERIZED
39	Breast tumor	None	None
30	Uterine myoma	None	None
24	Strabismus	1.2×10^4	1.8×10^3
6	Acute polio.	None	None
23	Chronic polio.	4.6×10^7	4.1×10^7
26	Acute polio.	None	None

*Bacteria per milliliter of urine.

Specimens were obtained by the two procedures within a period of a few hours in all 42 patients, and bacterial colony counts were performed. It can be seen from Table II that, of 81 pairs of voided and catheter specimens, 17 pairs were sterile. Among 28 pairs of specimens in which the catheterized specimen was sterile, 11 (37 per cent) of the corresponding clean voided specimens revealed bacterial growth. However, 9 of these 11 showed less than 10^5 (100,000) bacteria per milliliter, the degree of bacteriuria which is generally considered indicative of active urinary tract infection.[4, 5, 14] Of 25 catheterized specimens with less than 10^5 bacteria per milliliter, the corresponding clean voided specimen contained less than 10^5 organism per milliliter in 23 or 92 per cent of the determinations. The difference between the colony counts of the two other voided specimens which contained more than 10^5 bacteria per milliliter and the corresponding catheterized specimen was less than tenfold in one instance and between tenfold and one hundred fold in the other. Of 28 paired specimens in which the catheterized specimen yielded 100,000 or more bacteria per milliliter, the corresponding clean voided specimen contained 100,000 or more bacteria per milliliter in every instance.

Among specimens which yielded bacterial growth, the difference between the colony counts of the paired specimens was less than tenfold in 46 instances, ten to hundred fold in 15, and one hundred fold, or more, in only 3 instances (Table III).

Comparative colony counts in 6 representative patients are shown in Table IV.

The long series of sterile specimens obtained by the clean voided technique in the patient depicted in Fig. 1 indicates the freedom from contamination possible with these procedures. It is to be noted that when the voided specimen revealed bacterial growth of 100,000 or more organisms per milliliter the corresponding catheterized specimen also revealed bacteriuria of this degree or greater. The development of a pure growth of enterococci in the urine for 3 consecutive weeks following catheterization in the fifteenth week was a frequently observed aftermath of this procedure.

Thus, when the voided specimen yields bacterial growth but contains less than 10^5 organisms per milliliter of urine, it may be presumed with fair certainty without obtaining a catheterized specimen that significant bacteriuria is not present. When more than 100,000 organisms per milliliter of urine are present in the voided specimen, the likelihood is great that a similar degree of bacteriuria will be found if a catheterized specimen is obtained (Table II).

Aerobacter aerogenes, B. proteus, and *Pseudomonas aeroginosa* were the strains found most commonly in specimens with high bacterial counts whereas *Alcaligenes faecalis* and coagulase-negative, nonhemolytic staphylococci, species usually considered as nonpathogenic in the urinary tract, were isolated most frequently from specimens with low bacterial counts.

Comparison of Bacterial Colony Counts With Presence of Bacteria on Gram-Stained Smear.—A gram-stained smear of a portion of uncentrifuged urine from each specimen was obtained throughout this study. In this study

84 per cent of urine specimens with 100,000 or more organisms per milliliter exhibited the presence of bacteria on the gram-stained smear. This correlation is illustrated by the patient shown in Fig. 1.

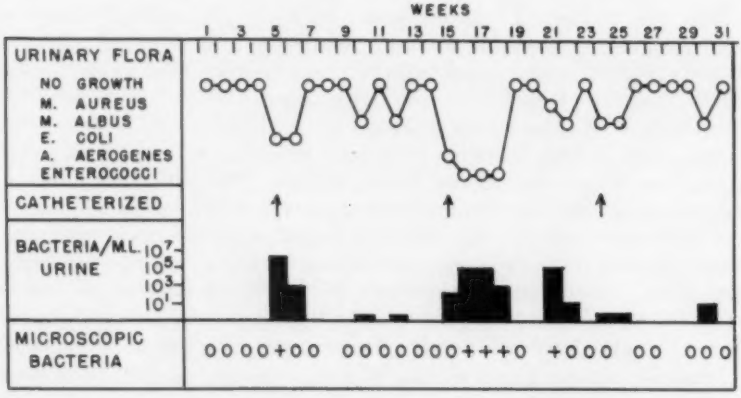

Fig. 1.—The evaluation of urinary tract infection in a 16-year-old girl with paralytic poliomyelitis by voided urine specimens and quantitative culture techniques.

DISCUSSION

Catheterization of the bladder, one of the most frequently employed procedures in medical practice, is commonly considered a harmless procedure. However, there is considerable evidence that there is no justification for the assumption that a catheter or other instrument introduced into the bladder passes through a bacteria-free passage. This often results in the development of cystitis which may be followed by pyelonephritis, a disease of undoubted gravity.

Although information on the microbial flora at all levels of the urinary tract is not available, it is now well known that the urethra may harbor bacteria.[7-10, 20, 21]

Guze and Beeson[11] have made interesting observations on the reliability and safety of bladder catheterization in regard to bacteriologic study of the urine. Urine specimens were obtained from women by direct needle aspiration of the bladder at laparotomy; shortly following this a second specimen was obtained by catheterization in the operating room under strict aseptic technique. Of the 12 urine specimens obtained by each technique, bacterial growth occurred in 4 of those obtained by urethral catheterization, whereas all those obtained by aspiration were sterile. Culture of the tip of the catheter after it had been inserted into the urethra of females carefully prepared in the operating room yielded bacterial growth in 6 of 13 cases. It is clear from this that a catheter or other instrument inserted into the bladder must traverse this flora. Furthermore, it is apparent that regardless of the care that is observed catheterization carries with it the danger of introducing infection into the bladder.

Clabaugh and Rhoads[12] found that contamination was reduced by obtaining bladder urine through a small rubber catheter inserted into the bladder through a large catheter inserted only into the urethra and not into the bladder.

Garrod[13] has shown that almost 20 per cent of women catheterized within the previous month developed urinary infection, whereas only 2 per cent of those not catheterized developed infection. The likelihood of inducing significant bacteriuria after a single catheterization in females has been found to be 2 to 4 per cent.[14, 22]

Kass and Schneiderman[16] demonstrated the ease with which bacteria gained entry into the urinary tract of patients with inlying catheters. This was carried out by placing a small amount of culture of *Serratia marcescens* to the periurethral epithelium of one female and two male patients with inlying catheters in the bladder. Within 1 to 3 days large numbers of the test organisms were recovered from the urine of these patients. It is suggested that the entry of the organisms into the urinary tract was effected via the fluid, probably composed of urine and exudate, that usually forms around the catheter.

Badal and associates[17] have shown that bacteriuria invariably follows within 72 hours the insertion of an indwelling catheter into the bladder. MacDonald and associates[18] found a substantial correlation between the use of indwelling catheters and high bacterial counts in the urine. Bladder catheterization has been shown to be an important influence in the development and severity of urinary tract infection.[5] Prophylactic antimicrobial therapy during bladder catheterization is generally ineffective and often results in the development of drug-resistant strains.[5, 15] Other workers report urinary infection almost inevitable after the use of an indwelling catheter. Furthermore, in the presence of established urinary tract infection, catheterization may be responsible for the introduction of drug-resistant microorganisms. Catheterization is also a serious cause of cross-infection in hospitals[30] and the frequent occurrence of bacteremia after all types of urethral instrumentation is well known.

On the other hand the results of this investigation indicate that the voided technique is a satisfactory method of obtaining urine specimens for culture in female patients. In another study from this clinic 207 of 1,182 consecutive cultures obtained by this technique were sterile. Proteus species and *A. aerogenes,* organisms commonly found in urinary tract infection, accounted for 68.6 per cent of the 1,213 strains isolated from the 975 positive cultures. Other gram-negative bacilli, enterococci, and coagulase-positive staphylococci, also well known etiologic agents of urinary infection, made up 22 per cent, whereas *Alcaligenes faecalis* and coagulase-negative staphylococci, frequent saprophytes of the urinary tract, constituted only 7.2 per cent. These latter two organisms as well as others usually considered as contaminants were isolated from catheter specimens with approximately the same frequency as from specimens obtained by the clean voided technique.[5]

Since cooperation of the patient is necessary to obtain satisfactory specimens by the clean voided method, the age of the patient is, or course, important. There was good correlation between the cultural results by the two techniques in the youngest patient in this study, a 1½-year-old girl. Further studies in this regard are in progress.

Kass[14] found the correlation between bacterial counts over 100,000 in clean voided and catheterized specimens from the same person to be over 95 per cent. Boshell and Sanford[19] also obtained excellent quantitative correlation between the results of clean voided and catheterized methods in females.

In evaluating cultures of the urine the possibility of false-positive results due to contamination by organisms introduced into the bladder urine during passage of a catheter or another instrument or from the normal urethral flora can never be eliminated positively. In many cases the patient is presumed to have a urinary tract infection if properly collected urine produces bacterial growth in the usual culture media. However, a "positive" urine culture may result even if only a few contaminating organisms have fallen into the urine. It has been shown by several workers that when bladder urine from patients proved to have acute pyelonephritis was examined quantitatively none contained less than 100,000 bacteria per milliliter.[4-6, 24] When female patients with no clinical evidence of urinary tract infection were catheterized their bladder urines were found to be free of bacteria in 26 per cent of cases, to contain less than 100 bacteria per milliliter in about 40 per cent more, and to contain less than 1,000 bacteria per milliliter in about an additional 30 per cent.[6] It also has been shown that pathologic findings compatible with pyelonephritis are much more common in patients with bacterial counts of 10^5 or greater per milliliter than in those without significant bacteriuria.[18] Thus, it is apparent that significant bacteriuria is present when bacterial counts are 10^5 or more per milliliter of urine and that values significantly lower than this usually represent contamination.[4, 25] Kass[6] has pointed out the circumstances which may exist when bacterial count below 10^5 ml. of urine are found in the presence of active pyelonephritis.

It has been shown that bacterial multiplication in urine is extremely rapid.[4] For this reason it is essential that specimens of the urine for quantitative evaluation be processed rapidly. They should be examined within an hour after collection or immediately refrigerated for subsequent study within 48 hours.

For practical clinical purposes contamination can be differentiated from infection by means of a gram-stained smear of the freshly collected uncentrifuged urine. Organisms were readily found in stained specimens of 84 per cent of urine samples containing 10^5 or more bacteria per milliliter. In other studies[5] from this clinic as well as the experience of others[4, 6] there was a high degree of correlation between the results of gram-stained smears and bacterial counts from the same specimen of urine.

The close qualitative and quantitative correlation between these two techniques and the relative infrequency of urinary tract saprophytes isolated

indicate that the clean voided technique is a reliable method, when carefully carried out, for the detection and evaluation of urinary tract infection in females. It is a particularly valuable technique when used in conjunction with colony counts and smears of the urine. It is strongly emphasized that the reliability of the results are dependent on the care with which the specimens are collected.

In view of the potential hazards of catheterization it is important that it be avoided whenever possible in patients particularly predisposed to urinary tract infections such as those with diabetes, paralytic poliomyelitis, and others with neurogenic or structural uropathies. In view of the presently inadequate methods of treatment of urinary tract infection, all efforts should be made to avoid any procedure capable of provoking such infection.

SUMMARY

In order to avoid the hazards of bladder catheterization, a method of collecting urine for culture in females by means of a clean voided technique was investigated. A comparison of 81 pairs of catheterized and voided urine specimens from 42 female patients, 34 of whom had paralytic poliomyelitis and 8 with no evidence of urologic disease or conditions predisposing to urinary tract infection, was made. Comparison of these paired samples from the same individual according to the species and number of bacteria isolated revealed that satisfactory urine specimens for culture can be obtained by the voided technique. When used in conjunction with colony counts or gram-stained smears of the urine, it is an adequate method of detecting significant bacteriuria in females. It is a particularly valuable procedure for screening for urinary tract infection, especially in patients predisposed to urinary infection and in whom catheterization is almost certain to produce urinary infection. The risks inherent in bladder catheterization should not be neglected and its use restricted to situations in which it is clearly indicated. To establish an accurate diagnosis of urinary tract infection determination of the number of bacteria present in the urine is as important as their identification.

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RADIOACTIVE VITAMIN B_{12} IN THE LIVER

I. Hepatic Uptake of $Co^{60}B_{12}$ in Liver Disease

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DETERMINATIONS by microbiologic techniques of the content of vitamin B_{12} in blood serum and in specimens of liver obtained by surgical or needle biopsy or post mortem have revealed disturbances of B_{12} metabolism in liver disease. Thus, in human viral hepatitis, vitamin B_{12} is discharged from destroyed liver tissue into the blood, and abnormally large amounts of bound vitamin B_{12} circulate in the blood plasma.[1-4] In experimental animals, in whom acute liver damage has been produced by carbon tetrachloride, the amount of vitamin B_{12} in the liver tissue decreases considerably, and that of the plasma rises sharply, owing to the discharge of vitamin B_{12} from the liver into the blood.[5, 6] Postmortem studies of patients who had died of cirrhosis of the liver demonstrated decreased B_{12} content of the liver[7] and often high vitamin B_{12} levels in the serum.[1-3, 8, 9] This may indicate either a decreased deposition of vitamin B_{12} or its increased discharge from the liver in this condition.

Consequently, it became of interest to approach this problem with an isotope technique, which made possible determinations of the deposition of B_{12} in the liver in vivo. By using vitamin B_{12} tagged with radioactive Co^{60}, such a study in the human liver becomes possible. Following the administration of $Co^{60}B_{12}$, the deposition and storage of vitamin B_{12} and its discharge from the human liver may be determined by the recording of the radioactivity on the surface of the liver with a scintillation probe, and quantitation of the radioactivity emanating from this organ.[10, 11]

MATERIAL AND METHOD

Thirty-six patients with liver disease were studied. These included 9 cases of virus and serum hepatitis; 9 of hepatic cirrhosis; 2 with leukemic infiltration of the liver; 2 with obstructive jaundice; 10 with primary or secondary malignancy of the liver, with or without jaundice; 1 of schistosomiasis of the liver; 1 with hypersplenism and hepatomegaly; and 2 with hepatomegaly of unknown origin. In each of these cases, liver function tests were performed and correlated with hepatic uptake measurements.

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The uptake of vitamin B_{12} by the liver was determined by external measurement of the radioactivity emanating from this organ by means of a scintillation counter with a 1 by 1½ inch sodium-iodide thallium crystal, applied directly without collimator to the cutaneous liver projections. The details of the method have been described in our earlier publications.[10-13] With the exception of 5 tests in which the counts were made on the third day by the accelerated method,[13] all measurements were performed between the seventh and the tenth day after oral administration of the tracer dose of 0.5 μg of radioactive vitamin B_{12}, which contained 0.45 μc of radioactive Co^{60}. Radioactive B_{12}* was given orally alone to 36 patients in 44 tests, and together with an intrinsic factor preparation from animal sources, standardized in USP antianemia units,† in 16 other tests. In 7 patients, the hepatic uptake of radioactivity was also measured after parenteral administration of 0.5 μg of sterile $Co^{60}B_{12}$, which contained 0.45 μc of Co^{60}. In all instances, the hepatic uptake was determined, and considered to be the average of three 5-minute counts taken over the anterior, anterolateral, and lateral projections of the liver, from which the background (usually 70 to 90 c.p.m. with our settings) was deducted. The result was finally calculated per 1 μc Co^{60}.

In 14 cases, vitamin B_{12} levels were determined by the *Ochromonas malhamensis*[14] and/or the *Euglena gracilis*[15] methods.‡

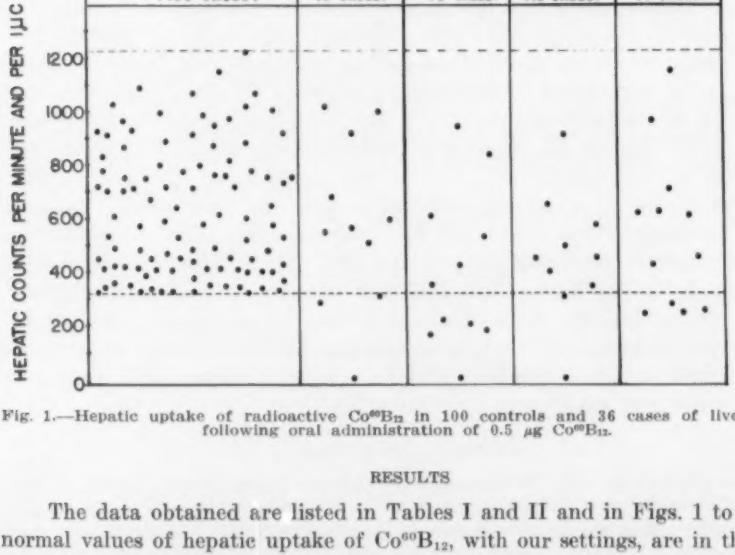

Fig. 1.—Hepatic uptake of radioactive $Co^{60}B_{12}$ in 100 controls and 36 cases of liver disease following oral administration of 0.5 μg $Co^{60}B_{12}$.

RESULTS

The data obtained are listed in Tables I and II and in Figs. 1 to 3. The normal values of hepatic uptake of $Co^{60}B_{12}$, with our settings, are in the range of 320 to 1,200 counts per minute per 1 μc Co^{60}, in which values of 320 to 400 counts per minute are considered low.[12]

*Generously supplied through the courtesy of Dr. N. S. Ritter and Dr. Charles Rosenblum of Merck & Company, Inc., Rahway, N. J.

†Generously supplied through the courtesy of Dr. K. W. Thompson, Medical Director and Vice-President of Organon, Inc., Orange, N. J.

‡These assays were performed through the courtesy of Dr. Herman Baker of the Department of Chemistry, Mount Sinai Hospital, New York, N. Y.

In 3 out of 36 instances of liver disease, the hepatic uptake of radioactive vitamin B_{12} was abolished entirely (Cases 1, 13, and 24). This was observed in 1 case of severe infectious hepatitis (in the acute stage of the disease), in a terminal Laennec's cirrhosis a few weeks before death, and in a patient with extensive metastatic carcinoma of the liver. In all remaining cases, the uptake by the diseased liver was preserved, but in 11 tests on 8 cases it was below the lower normal level (Fig. 1).

Impairment of the uptake of B_{12} by the liver was noted in 2 of 9 cases of infectious hepatitis (Cases 5 and 8). The abolished B_{12} uptake by the liver in the acute stage of hepatitis (Case 1), reverted to normal with improvement of liver function. There was often a lack of relationship, however, between the uptake of the vitamin by the liver and the extent of liver damage as estimated from the clinical symptomatology and biochemical liver function tests. This is exemplified by the normal uptake of $Co^{60}B_{12}$ and abnormal liver function tests in Cases 2, 3, 4, 6, 7, and 9, and the normal B_{12} hepatic uptake and high B_{12} level in the blood in Case 4.

In 4 of 9 cases of liver cirrhosis, impaired uptake of radioactive vitamin B_{12} by the liver was manifest, especially in 3 severely ill patients shortly before death (Cases 11, 13, and 17). In the fourth case (10), it became normal, with improvement of the general condition. In 5 other cases of liver cirrhosis, no impairment of hepatic uptake of vitamin B_{12} was evident. In 1 of these cases (14), the test was performed one week before the death of the patient. In 1 of 2 cases of jaundice due to calculous obstruction of the common duct, hepatic uptake was impaired, while in the other it was normal.

In malignant lesions of the liver, the hepatic deposition of radioactive vitamin B_{12} was completely abolished in 1 case out of 10, and decreased in another. In the remaining 8 cases, often in spite of very extensive infiltration of the liver, the hepatic uptake of radioactive vitamin B_{12} did not show any deviation from the norm. In 2 cases of leukemic involvement of the liver with jaundice, hepatic uptake was initially within normal limits. When reviewed later, 3 months before death, one showed a definite decrease. In 2 other cases of hepatomegaly of unknown origin, and in one case of schistosomiasis of the liver, the uptake was within normal limits, but decreased in one case of hypersplenism.

The blood level of vitamin B_{12} was increased above normal in 2 out of 4 cases of hepatitis, in 1 out of 5 cases of liver cirrhosis, and in 1 out of 4 cases of metastatic carcinoma of the liver; was normal in 2 cases of hepatitis, 3 cases of liver cirrhosis, 2 cases of metastatic carcinoma of the liver, and in 1 case of hypersplenism; and was decreased in 1 case of liver cirrhosis and 1 case of metastases to the liver. These data, as well as the results of measurements of hepatic uptake of $Co^{60}B_{12}$, indicate that, in many cases of liver disease, no abnormalities in vitamin B_{12} metabolism can be detected by these methods.

In 15 cases of liver disease, measurements of hepatic uptake were also made after the addition to the $Co^{60}B_{12}$ tracer of a potent intrinsic factor

TABLE I. HEPATIC UPTAKE OF $Co^{60}B_{12}$, VITAMIN B_{12} LEVELS IN BLOOD AND

CASE NO.	PATIENT, SEX, AND AGE	DIAGNOSIS	TEST AND TIME INTERVAL	HEPATIC UPTAKE OF $Co^{60}B_{12}$ IN C.P.M. PER 1 μc Co^{60} FOLLOWING ADMINISTRATION OF: $Co^{60}B_{12}$ ALONE 320-1,200*	$Co^{60}B_{12}$ + INTR. FACT 320-1,200*	$Co^{60}B_{12}$ I.M. 500-1,200*
1	M. S., m., 26	Virus hepatitis	a. —	0		
			b. 1 wk.	558		
			c. 2 wk.		520	
			d. 3 wk.	549		
			e. 4 wk.			1,009
2	B. B., f., 44	Virus hepatitis	a. —	680		
			b. 1 wk.		971	
3	R. McD., f., 44	Virus hepatitis	a. —	1,026		
			b. 2 wk.		1,050	
4	H. R., m., 22	Virus hepatitis	a. —	1,002		
5	W. M., m., 19	Virus hepatitis	a. —	317†		
6	J. R., m., 19	Virus hepatitis	a. —	919		
7	E. H., f., 24	Virus hepatitis	a. —	596		
			b. 1 wk.		317	
8	L. F., f., 26	Virus hepatitis	a. —	283		
			b. 1 wk.		376	
9	V. A., m., 33	Serum hepatitis	a. —	525		
10	F. D., m., 50	Liver cirrhosis	a. —	207		
			b. 1 wk.	423		
			c. 2 wk.		625	
			d. 3 wk.			1,003
11	S. P., f., 34	Liver cirrhosis	a. —	166		
12	E. C., f ., 34	Liver cirrhosis	a. —	840		
			b. 1 wk.		665	
13	P. Z., f., 48	Liver cirrhosis	a. —	286		
			b. 1 wk.		263	
			c. 4 wk.		0	
			d. 5 wk.	0		
			e. 7 wk.			525
14	W. A., m., 40	Liver cirrhosis	a. —	608		
			b. 2 wk.		647	
15	N. D., m., 40	Liver cirrhosis	a. —	943†		
16	P. P., f., 69	Liver cirrhosis	a. —	533		
17	D. W., m., 29	Liver cirrhosis	a. —	228		
			b. 3 wk.		230	
18	B. G., m., 68	Liver cirrhosis; macrocytic anemia	a. —	356		
			b. 1 wk.		485	
19	S. G., f., 39	Hypersplenism with hepatomegaly	a. —	278		

*These figures represent normal values.
†Hepatic uptake measured by the accelerated method.[12]

LIVER FUNCTION TESTS IN 19 PATIENTS WITH HEPATITIS AND LIVER CIRRHOSIS

B_{12} IN SERUM ($\mu\mu$g/ML.)		LIVER FUNCTION TESTS						
OCHROMONAS 250-800*	E. GRACILIS 300-800*	ICTERIC INDEX	BILIRUBIN (MG. %)	CEPHALIN FLOCCULATION	THYMOL TURBIDITY	ALKALINE PHOSPHATASE	CHOLESTEROL TOTAL/ESTERS (MG. %)	ALBUMIN/GLOBULIN (GM. %)
	940							
		30	5.9	4+	6	30.6	184/100	3.0/4.0
		6	0.9	3+	5	12.0	200/128	4.3/3.0
		7	0.5	2+	4	14.5	180/119	4.0/2.5
		5	0.6	2+	4	11.4	197/119	5.6/1.8
			28.8	3+	2	7.3	172/ 59	1.9/3.2
			17.6			4.9	178/ 82	
			22.6	2+		20.6	298/143	3.7/3.0
			9.6	1+		2.7		3.5/2.9
	867			3+				3.2/3.1
340	350		2.3	3+	1		94/ 59	
121			2.9	3+		8.8	180/ 96	3.9/2.1
		7	0.5	1+	9	25.5	221/156	4.4/2.9
		3	0.3	1+	9	16.4	357/240	4.3/3.0
		60	16.0	4+	6	27.1	189/ 81	3.7/3.0
		10	1.6	1+	5	15.8	234/167	4.2/2.5
		18		3+				
			3.5	3+	8			2.0/3.8
		15	23.7	1+	2	31.7	164/ 53	3.0/2.6
				4+	34	7.6		2.8/3.4
			18.0	2+	3	6.8	340/245	3.8/1.8
	920							
		12	1.8	3+	6	21.8	108/ 75	2.3/3.9
			3.4	4+	2	4.2	105/110	2.0/4.3
	424							
10	10			3+		4.5	187/134	3.5/3.4
			0.6				157/ 82	2.4/2.3
350	579							
		22		3+				2.6/4.0
80	278							
			3.0	3+				2.9/3.8
		5	0.5	4+	8	11.4	136/ 99	3.8/3.6

TABLE II. HEPATIC UPTAKE OF $Co^{60}B_{12}$, VITAMIN B_{12} LEVELS IN BLOOD, AND LIVER FUNCTION AND VARIOUS OTHER

CASE NO.	PATIENT, SEX, AND AGE	DIAGNOSIS	TEST AND TIME INTERVAL	HEPATIC UPTAKE OF $Co^{60}B_{12}$ IN C.P.M. PER 1 μC Co^{60} FOLLOWING ADMINISTRATION OF: $Co^{60}B_{12}$ ALONE 320-1,200*	$Co^{60}B_{12}$ + INTR. FACT. 320-1,200*	$Co^{60}B_{12}$ I.M. 500-1,200*
20	A. W., f., 64	Calculous obs. of common duct with jaundice	a. —	624		
			b. 1 wk.		670	
			c. 2 wk.			732
			d. 3 wk.	714		
			e. 4 wk.	457		
21	G. LaP., f., 61	Calculous obs. of common duct with jaundice	a. —	245		
			b. 2 wk.	262		
			c. 3 wk.			653
22	M. I., f., 70	Ca. gall bladder; met. ad portam hepatis; obs. jaundice	a. —	917		
23	G. B., m., 43	Ca. stomach; liver metastases	a. —	311		
			b. 1 wk.		280	
24	R. M., f., 62	Ca. rectum; liver metastases	a. —	0		
			b. 1 wk.			0
25	O. A., m., 54	Ca. pancreas; met. ad portam; hepatitis; obs. jaundice	a. —	345		
26	J. R., m., 55	Ca. stomach; liver metastases	a. 2 wk. before death	446		
27	D. P., f., 67	Ca. stomach; liver metastases	a. —	510†		
28	J. V., m., 53	Ca. stomach; liver metastases; obs. jaundice	a. 5 wk. before death	658†		
29	F. T., m., 67	Ca. stomach; liver metastases; obs. jaundice	a. —	401		
30	A. T., f., 67	Ca. bronchi; liver metastases	a. —	578		
31	A. McC., f., 52	Hepatoma	a. —	555		
32	A. K., f., 40	Myelogenous leukemia; jaundice	a. —	423		
			b. 1 wk.		347	
			c. 2 wk.			583
33	E. G., f., 81	Lymphocytic leukemia; hepatomegaly; jaundice	a. —	616		
			b. 1 wk.	243		
34	D. A., f., 30	Schistosomiasis of liver	a. —	965		
			b. 1 wk.		531	
35	M. M., f., 46	Hepatomegaly of unknown origin	a. —	1,166†		
36	A. C., m., 64	Hepatomegaly of unknown origin	a. —	625		

*These figures represent normal values.
†Hepatic uptake measured by the accelerated method.[13]

TESTS IN 17 PATIENTS WITH OBSTRUCTIVE JAUNDICE, METASTATIC MALIGNANCY OF THE LIVER, DISEASES OF THE LIVER

B_{12} IN SERUM ($\mu\mu$G/ML.)		LIVER FUNCTION TESTS						
OCHROMONAS 250-800*	E. GRACILIS 300-800*	ICTERIC INDEX	BILIRUBIN (MG. %)	CEPHALIN FLOCCULATION	THYMOL TURBIDITY	ALKALINE PHOSPHATASE	CHOLESTEROL TOTAL/ESTERS (MG. %)	ALBUMIN/GLOBULIN (GM. %)
		14	1.9		2	28.4	335/235	3.6/2.1
		5	0.6		1	22.3	269/202	4.4/1.8
		30	4.0		1	97.9	360/279	4.2/3.0
		15	1.8		1	34.3	432/333	4.2/2.8
			8.8			129.0	423/340	3.3/3.0
650	600		1.5			62.0		
	880	3	0.2	2+	2	10.5	280/176	3.9/2.6
		60	12.6	1+	1	71.0	319/221	
						33.5		2.5/3.4
				3+	2	11.2	272/127	2.8/3.1
330	553	113	35.0	–	3.6	32.6	324/121	2.9/2.2
44	60	46		1+		36.0		2.6/2.8
		6	1.3	2+	1	118.9	189/ 95	2.8/2.3
		9	0.8	3+	2	39.3	168/ 92	
			1.3		8	24.8	185/ 93	4.1/3.9
			1.6	4+	1.7	12.6	197/108	3.4/3.3
114	205		2.0	2+		3.8	136/ 60	3.0/2.8
		8		1+		4.0		

concentrate* (Fig. 2). In 1 out of 5 tests on 4 individuals it was possible, by addition of the intrinsic factor preparation, to raise the decreased hepatic uptake of B_{12} slightly. In other cases, most of them with a normal hepatic uptake of B_{12}, no enhancement of the hepatic uptake of B_{12} was observed after the addition of intrinsic factor preparation.

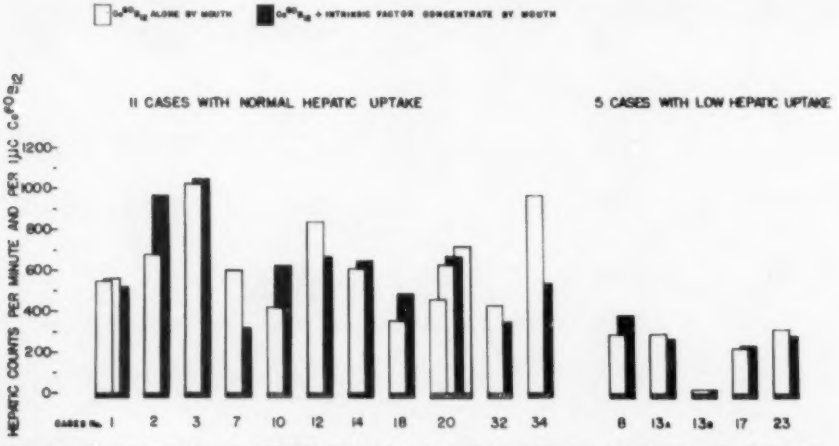
Co60B12 ALONE BY MOUTH
Co60B12 + INTRINSIC FACTOR CONCENTRATE BY MOUTH
11 CASES WITH NORMAL HEPATIC UPTAKE
5 CASES WITH LOW HEPATIC UPTAKE
HEPATIC COUNTS PER MINUTE AND PER 1μC Co60B12
1200
1000
800
600
400
200
0
CASES No. 1 2 3 7 10 12 14 18 20 32 34 8 13A 13B 17 23

Fig. 2.—Hepatic uptake of radioactivity in 15 cases of liver disease following oral administration of 0.5 μg $Co^{60}B_{12}$, alone and together with intrinsic factor concentrate.

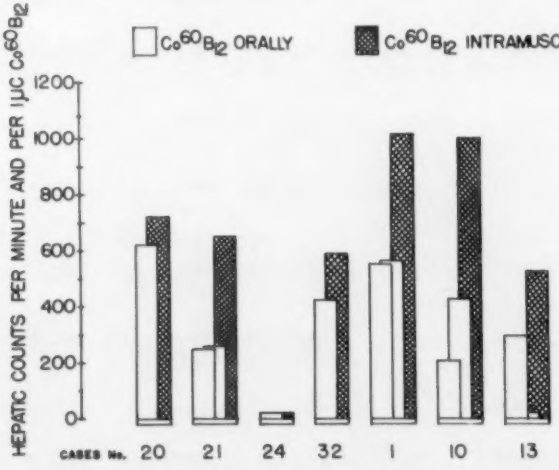

Fig. 3.—Hepatic uptake of radioactivity in 7 cases of liver disease following oral and intramuscular administration of 0.5 μg $Co^{60}B_{12}$.

In 7 cases, the hepatic uptake of vitamin B_{12} was also determined following parenteral administration of a similar tracer dose of $Co^{60}B_{12}$ (Fig. 3). It

*The intrinsic factor preparation used did not contain any appreciable amount of B_{12} on microbiologic assay, and was used at a dose of 62 mg., equivalent to dose contained in 2 USP units of oral antianemia preparation (Organon).

was entirely abolished in 1 case of metastatic carcinoma of the liver in the terminal stage. It was within normal limits in 6 other cases. The hepatic uptake of $Co^{60}B_{12}$, after parenteral administration, was always higher than after oral ingestion of a similar dose of the tracer, either alone or together with intrinsic factor. This is in agreement with previous observations regarding the limited efficiency of absorption of B_{12} in the intestines of human beings.[16] In 1 case of advanced Laennec's cirrhosis, in which the hepatic uptake following oral administration of $Co^{60}B_{12}$ alone was entirely abolished, a similar dose of $Co^{60}B_{12}$, administered parenterally 10 days before the patient's death, resulted in a positive uptake of radioactivity by the liver. This was at the lower limit of the norm, which, with our settings on parenteral administration, is 500 c.p.m. per 1 μc Co^{60}.[16]

DISCUSSION

In the majority of cases of liver disease, the hepatic parenchyma preserves the ability to take up vitamin B_{12} from the portal and systemic circulations, even when biochemical tests show a rather severe impairment of liver function. In about one-third of the cases of liver disease, however, the ability of the liver to take up B_{12} from the portal circulation is impaired. In the present work, some preliminary results of which were reported at an earlier date,[17] measurements of the hepatic uptake of radioactive B_{12} were made following administration of a small tracer dose of 0.5 μg of B_{12}. This represents only about 1/1,000 to 1/2,000 of the total amount of vitamin B_{12} stored in the liver tissue under normal conditions. It is quite probable, therefore, that if the total capacity of the liver for vitamin B_{12} were to be much decreased as a result of destruction of the liver parenchyma by hepatitis, malignant infiltration, or fibrosis, there would still remain some residual capacity for an uptake of 0.5 μg of B_{12} by the liver tissue. A tolerance test, performed with a much larger dose of B_{12}, might show impairment under these conditions, while the same test, performed with a small dose of 0.5 μg, would not reveal any abnormality. The recent observations of Baker and associates[18] point to this possibility. Safety requirements preclude similar isotope tolerance tests.

In 3 of 4 cases of liver disease, the addition of intrinsic factor preparations did not increase the impaired hepatic uptake of vitamin B_{12}. This suggests that the impaired deposition of B_{12} in liver disease, if present, is not due to the decreased output of intrinsic factor in the stomach. It stands to reason that the lowered hepatic uptake of B_{12} in liver disease is due to the impaired ability of the diseased liver cells to take up vitamin B_{12} from the portal circulation.

The decreased hepatic uptake of vitamin B_{12} in some cases of liver cirrhosis, especially in the terminal stage, may contribute to the increased content of B_{12} in the serum in some of these cases.[1-3, 8, 9] Our results suggest that impaired uptake of B_{12} by the diseased liver may result in the passage of an excessive amount of B_{12}, ingested in food or in medication, from the portal circulation into the peripheral blood. This would result in an increased B_{12} blood level in these cases. The increased B_{12} blood level and decreased B_{12}

content of the liver in obstructive jaundice,[19, 21] leukemic liver,[19] extensive primary or metastatic carcinomatous invasion of the liver,[19, 20] and infectious hepatitis[1, 2, 19] may be also due, in part, to a similar mechanism.

It appears from our data that in evaluating the disturbances of the metabolism of vitamin B_{12} in liver disease, one must also take into consideration the impaired ability of the diseased liver to deposit vitamin B_{12} brought to it by the portal circulation.

SUMMARY AND CONCLUSIONS

Measurements of the uptake of vitamin B_{12} by the liver were performed in 36 cases of liver disease following oral administration of 0.5 μg (0.45 μc) radioactive $Co^{60}B_{12}$, alone or with potent intrinsic factor preparation. In 11 of these cases, including infectious hepatitis, cirrhosis, primary or secondary liver malignancy, leukemic infiltration of the liver, obstructive jaundice, and hypersplenism, more or less pronounced impairment of the uptake of vitamin B_{12} by the liver was observed.

The impairment of the ability of the liver tissue to take up vitamin B_{12} from the portal circulation may be an additional contributory factor in causing high B_{12} blood levels and low B_{12} content of the liver tissue in some cases of hepatitis, liver cirrhosis, and malignant liver infiltration.

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RADIOACTIVE VITAMIN B_{12} IN THE LIVER

II. Hepatic Deposition, Storage, and Discharge of $Co^{60}B_{12}$ in Dogs

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With the Technical Assistance of Roger W. Laughton, A.B., Herman Schaffer, B.A., and Gabriel Schwartz, B.S.

THE pivotal role of the liver in the metabolism of vitamin B_{12} is widely recognized,[1] but the kinetics of the hepatic uptake of this vitamin and its storage and discharge from this organ are incompletely understood. In the rat, the kidneys are the main organs in which B_{12} is stored.[2-8] In man,[9] guinea pig, hamster, and mouse,[8] the liver is the main depository for this vitamin.

Experimental studies on animals have shown that the liver retains administered vitamin B_{12} for very long periods of time.[5, 6] In earlier studies with radioactive vitamin B_{12}, we demonstrated that the same is true for human beings.[9] Using the method of surface counting of the cutaneous projections of the liver with a scintillation detector, we found that 2 to 3 months after parenteral administration of a tracer dose of radioactive $Co^{60}B_{12}$, the counts over the liver were still 65 to 86 per cent of the initial values,[9] and that 8 months after injection, over 35 per cent of the radioactivity was still retained in the liver.[10] These findings have been confirmed and extended by Meyer and his collaborators.[11, 12] Schloesser and Schilling[13] also used the same technique and concluded that the biologic half-life of B_{12} in the liver was, on the average, about one year in man. All these findings confirmed the earlier observation of Smith and associates,[14] made post mortem on a patient with pernicious anemia, who retained a hepatic deposit of radioactive B_{12} for months.

To what extent does the radioactivity in the liver, after administration of $Co^{60}B_{12}$, represent the content of radioactive B_{12} in this organ? There is circumstantial evidence that it truly reflects the hepatic storage of labeled vitamin B_{12}, and is not derived from the labeled cobalt[60] atom detached from the B_{12} molecule and stored in the reticuloendothelial apparatus of the liver. The investigations of Meyer and associates,[12] Schloesser and Schilling,[13] and our own earlier work[9] give support to this conclusion. Moreover, in view of the preferential localization of vitamin B_{12} microbial activity in the mitochondrial fraction of the liver cells,[15] it is improbable that the cobalt atom is detached from

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it and stored in the reticuloendothelial apparatus. This would have caused loss of microbial B_{12} activity of the mitochondria.

In recent collaborative work with Weston and Wolf,[16] we obtained direct evidence that most of the radioactivity in the liver derives from storage of microbiologically active $Co^{60}B_{12}$ in this organ. Seven months after injection of $Co^{60}B_{12}$ into a dog, its liver was removed, processed by standard cyanide nitrite treatment, and extracted with benzyl alcohol, ethyl ether, and methanol. The radioactivity and microbiologic B_{12} activity of the extract was determined on descending paper chromatograms in secondary butanol saturated with water and with 0.5 per cent KCN added. No evidence was obtained for the existence of a microbiologically active compound containing radioactive Co other than cobalamin, and up to 63.7 per cent of the radioactivity of the extract was located in the cyanocobalamin zone of the chromatogram.

In the liver, B_{12} is bound to polypeptides of the hepatic cells,[17, 18] and this binding is apparently much looser than is the case with serum proteins.[19] Because of this loose binding, one would expect that B_{12} would be given up by the liver tissue more readily than by the serum proteins. This, however, is not in accord with the long hepatic storage time of B_{12}. The present investigation has been undertaken in order to collect more information as to the mechanisms regulating the hepatic storage and discharge of vitamin B_{12}.

MATERIAL AND METHODS

Eight medium-sized dogs (13.8 to 23.0 pounds in weight) were studied in pairs. Three pairs were given radioactive $Co^{60}B_{12}$ intramuscularly, by a single injection in the hind leg, in doses of 0.1, 0.15, and 0.6 μg, respectively, per pound of weight. Two other dogs received radioactive B_{12} orally in a dose of 0.12 and 0.15 μg, respectively, per pound of weight. One of these latter dogs died of internal hemorrhage after the first surgical biopsy of the liver, while the other lived until the end of the study, 220 days. The total oral dose was divided into 5 equal daily doses to enhance the intestinal absorption of B_{12}. The specific activity of $Co^{60}B_{12}$ used was 1 μc per 1 μg of material.* All animals were kept on the same standardized meatless "dog food complete ration" for the full duration of the observation period. In spite of the content of meat meal and alfalfa meal in the ration, this dietary regimen resulted in the development of nutritional vitamin B_{12} deficiency in our dogs. This was shown by a very low B_{12} content of the liver at the conclusion of the investigative period (see the following).

External Counting of the Hepatic Radioactivity.—Following parenteral or oral administration of radioactive B_{12}, external surface counting was performed. A scintillation detector with a 1 by 1½ inch sodium-iodide thallium crystal was used. Counts were taken over the 3 cutaneous projections of the liver (anterior, anterolateral, and lateral) for 10 minutes each. The reproducibility of the counts was ascertained by painstaking positioning of the dogs under anesthesia and by marking the areas of the skin with indelible dye. The statistical significance of the counts was similar to that reported in previous work.[9] The hepatic counts were averaged, the background was deducted, and the counts were corrected for physical decay of the Co^{60} and efficiency of the scaler by using dried B_{12} standards containing 0.01 μc of Co^{60}. The standards were prepared by drying, under infrared light, 0.01 μc aliquots of diluted samples of $Co^{60}B_{12}$ of the same batch which had been administered as a tracer. The correction factor was calculated from quadruple measurements of the two

*Generously supplied by Dr. Nathaniel Ritter and Dr. Charles Rosenblum of Merck & Company, Inc., Rahway, N. J.

standards before and after conclusion of the counts. The counting procedure was repeated at various time intervals. The period of observation for each of the dogs ranged from 205 to 302 days.

Liver Biopsies and Measurements of Radioactivity in the Organs.—After most of the surface countings, a surgical biopsy of the liver was performed, one or two specimens being taken each time. A total of 30 biopsies from the right and left lobes were performed. The size of the biopsy specimens varied from 0.45 to 3.55 Gm. (average: 1.24 Gm.). At about 7 to 10 months from the start of the experiment, the dogs were sacrificed, the entire liver removed, and the total radioactivity of this organ measured. In 2 dogs the radioactivity of several internal organs was measured as well. The liver, as well as the other organs (kidneys, spleen, pancreas, small intestine, lungs, and uterus) obtained by surgical biopsy or after sacrifice of the animal, were thoroughly washed with a stream of water, dried with filter paper, and homogenized in a Waring Blendor. A small amount of water was added, but no additional carrier was used in view of the large amounts of nonradioactive B_{12} contained in the organs studied, especially in the liver, kidneys, and pancreas. The 5 ml. aliquots of homogenates from the various organs were counted in vials of 6 ml. capacity, and set with a tight fit into the well of a heavily shielded well counter equipped with a large well-shaped NaI thallium crystal. Before the tissue homogenates were counted, similar vials containing 5 ml. of water were counted under the same conditions and the counts were deducted from the sample as a blank background. In addition, 3 liquid standards containing 0.01 μc of the same batch of $Co^{60}B_{12}$ dissolved in 5 ml. of water, were placed in the same-sized vials, then counted and averaged each time after counting of the tissues. The radioactivity content of the tissues was corrected for physical decay of the Co^{60} and efficiency of the scaler by the use of these standards and then calculated per 1 Gm. of wet weight of the tissues. Any further reference in this paper to the decay of hepatic radioactivity will refer exclusively to the biologic discharge of $Co^{60}B_{12}$ from the liver.

RESULTS

Hepatic Radioactivity as Determined by Surface Counting.—Results of the surface counting of the cutaneous liver projections of all 8 dogs are drawn on semilogarithmic paper in Fig. 1. There was an excellent duplication of results for each pair of dogs.

The slopes of the semilogarithmic tracings in Fig. 1 reflect the biologic decay (i.e., rate of discharge) of $Co^{60}B_{12}$ from the liver of the 8 dogs as determined by surface counts over the liver. The beginning of the fourth week after administration of $Co^{60}B_{12}$ was taken as the starting point for the calculation of the biologic half-life of B_{12} in the liver. During the first 3 weeks, the uptake of B_{12} by the liver is still in progress, as shown by our previous observations[9] and comparison of the hepatic counts in the dogs on the first, seventh, and twenty-third days after injection of $Co^{60}B_{12}$. This is probably due to the prolonged time needed for equilibration of the injected $Co^{60}B_{12}$ with the total nonradioactive B_{12} pool contained in the vascular and extravascular spaces of the body and the relocation of the injected $Co^{60}B_{12}$ between various tissues.[9]

The semilogarithmic tracings of hepatic surface counts do not follow a straight line, but show a much steeper slope at the onset than later. This is best seen in the dogs injected with moderate doses of radioactive B_{12} (Dogs 433, 435, 436, and 437), or in the one to whom radioactive B_{12} was given by mouth (438).

The biologic half-life of $Co^{60}B_{12}$ in the liver of 6 dogs, when calculated from the beginning of the fourth week, ranges from 65 to 113 days, depending on the dose of $Co^{60}B_{12}$ injected (Table I and Fig. 1). The slope of radioactivity counts over the liver is less abrupt, and the biologic half-life of $Co^{60}B_{12}$ in the liver is longer following the administration of a larger amount of this

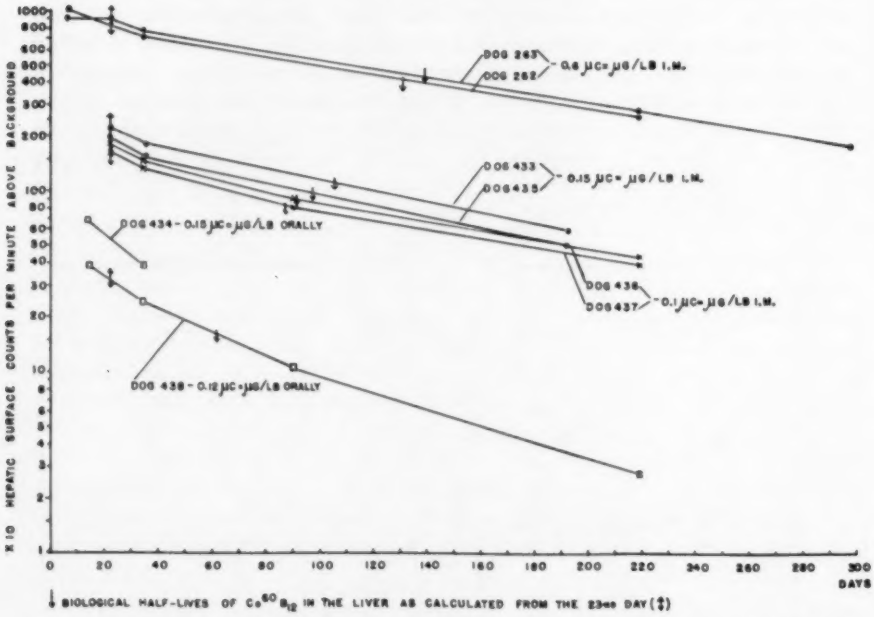
X 10 HEPATIC SURFACE COUNTS PER MINUTE ABOVE BACKGROUND
DOG 263
DOG 262
0.6 μC = μG/LB I.M.
DOG 433
DOG 435
0.15 μC = μG/LB I.M.
DOG 434 - 0.15 μC = μG/LB ORALLY
DOG 436
DOG 437
0.1 μC = μG/LB I.M.
DOG 438 - 0.12 μC = μG/LB ORALLY
DAYS
BIOLOGICAL HALF-LIVES OF Co60 B12 IN THE LIVER AS CALCULATED FROM THE 23rd DAY (↕)

Fig. 1.—Hepatic surface counts in 8 dogs followed up to 10 months after parenteral or oral administration of radioactive $Co^{60}B_{12}$.

TABLE I. DEPENDENCE OF BIOLOGIC HALF-LIFE OF $Co^{60}B_{12}$ IN THE LIVER FROM THE DOSE OF $Co^{60}B_{12}$ AND ITS ROUTE OF ADMINISTRATION

DOG NO.	DOSE OF $Co^{60}B_{12}$* AND ROUTE OF ADMINISTRATION	HEPATIC COUNT ABOVE BACKGROUND IN C.P.M. ON DAY 23	DAY ON WHICH HEPATIC COUNT WAS ½ THAT OF DAY 23	BIOLOGIC HALF-LIFE OF $Co^{60}B_{12}$ IN THE LIVER IN DAYS
438	0.12 μg orally	320	63	40
437	0.1 μg I.M.	1,680	88	65
436	0.1 μg I.M.	1,835	92	69
435	0.15 μg I.M.	1,980	98	75
433	0.15 μg I.M.	2,300	106	83
263	0.60 μg I.M.	8,545	132	109
262	0.60 μg I.M.	8,708	140	117

*Doses given in micrograms per pound of body weight.

vitamin than after a lesser amount. With the oral dose of 0.12 μg B_{12} per pound, the deposition of radioactivity in the liver is much below that after a similar dose administered parenterally, and the biologic half-life amounts to only 40 days.

A good correlation was observed between the amount of radioactive B_{12} injected and the hepatic radioactivity, as determined by surface scintillation counting of the cutaneous projections of the liver. Two dogs, injected with 0.6 μg of radioactive B_{12} per pound of weight, had on the average about 4 times as much radioactivity over the liver at the onset of the experiments as 2 others into whom a lesser dose, amounting to one-quarter (0.15 μg per pound) as much, had been injected. Still lower radioactivity over the liver was shown in 2 other dogs who received 0.1 μg of B_{12} per pound of weight by injection. The least hepatic radioactivity was found in dogs who received $Co^{60}B_{12}$ orally in doses of 0.12 and 0.15 μg, respectively, per pound of weight. When the hepatic radioactivity is calculated per pound of weight of the animal and per microcurie of radioactive B_{12} injected, the hepatic surface counts are similar for dogs who had received 0.15 and 0.6 μg of $Co^{60}B_{12}$ per pound of weight—1,430 and 1,438 counts per minute.

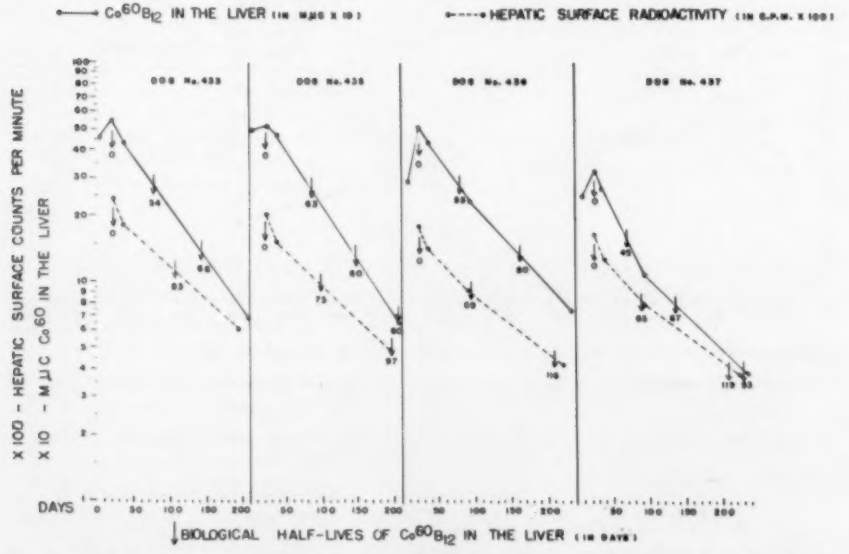
Co60B12 IN THE LIVER
HEPATIC SURFACE RADIOACTIVITY
DOG No. 433
DOG No. 435
DOG No. 436
DOG No. 437
X 100 - HEPATIC SURFACE COUNTS PER MINUTE
X 10 - MμC Co60 IN THE LIVER
DAYS
BIOLOGICAL HALF-LIVES OF Co60B12 IN THE LIVER

Fig. 2.—Hepatic storage and discharge of $Co^{60}B_{12}$ in 4 dogs as determined by surgical biopsies and hepatic surface counting.

In Fig. 2, the radioactivity data obtained by surface counting of the cutaneous liver projections in 4 dogs are correlated with the radioactivity of the homogenized liver specimens obtained by surgical biopsy. There is a good parallel between the hepatic surface radioactivity, determined by surface scintillation counting of the cutaneous projections of the liver, and the content of radioactive B_{12} in the liver, determined by consecutive liver biopsies. With the progress of time, however, recording of the hepatic surface radioactivity gives a higher count per unit of Co^{60} content in the liver than at an earlier stage

of the observations. This is shown by all 4 experiments traced in Fig. 2. While this ratio, at the beginning of the experiment, amounted to about 2,000 to 2,500 counts per 0.4 to 0.6 μc in the liver (i.e., about 4,500 c.p.m. per 1 μc, on the average), there were about 500 to 600 counts per minute per 0.05 to 0.1 μc Co^{60} at the end of the experiments. This averages about 7,300 c.p.m. per 1 μc Co^{60} in the liver. The reason for this finding may be a change in the geometry of the liver as a result of the repeated liver biopsies and loss in weight of the animals following multiple laparotomies. A closer approximation of the liver to the skin would result which would cause a relatively higher surface radioactivity over the liver.

Hepatic Radioactivity as Determined by Liver Biopsies.—It is evident, from the data listed in Table II, that the results of biopsies taken from the left and right sides of the liver differ not more than ±10 per cent (with the exception of Dog 435, where the difference amounted to almost 30 per cent).

In all instances, the radioactivity in the liver specimens taken on the twenty-third day was higher than in those taken on the first or seventh day following parenteral administration of radioactive $Co^{60}B_{12}$. With this maximum value taken as 100 per cent, the content of radioactivity in the liver on the first and seventh days was in the range of 66.2 and 92.3 per cent (see Table II). The progressive discharge of radioactive B_{12} from the liver causes the decrease of this value at the end of the seventh to eighth month to 11.4 to 14.5 per cent. The data listed in Table II indicate that, on the twenty-third day, the total radioactivity of the liver at its peak value amounts to 17.4 to 24.7 per cent of the total dose of $Co^{60}B_{12}$ injected. With the gradual discharge of this material from the liver after the seventh to eighth month, this percentage decreases to 2.1 to 3.3 per cent.

The biologic half-life of $Co^{60}B_{12}$ in the liver, calculated from the radioactivity of the biopsy specimens and the liver weight on autopsy, shows a pattern similar to that calculated from hepatic surface radioactivity. There is a progressive prolongation of the half-life time in most instances (with the exception of Dog 435).

The radioactivity of the liver biopsy specimens of dogs who obtained $Co^{60}B_{12}$ orally was less than one-tenth that following a similar dose given parenterally. A good comparison is provided by data obtained on the thirty-fifth day after administration of $Co^{60}B_{12}$. In 4 dogs who received 0.1 or 0.15 μg $Co^{60}B_{12}$ per pound of weight intramuscularly, the hepatic radioactivity amounted to 14.5 to 22.3 per cent (average, 18.3 per cent) of the total radioactivity injected. In 2 dogs who received 0.12 and 0.15 μg, respectively, per pound of weight orally, the hepatic radioactivity was only 1.6 to 1.8 per cent on the same (the thirty-fifth) day.

The half-life of $Co^{60}B_{12}$ in the liver, as calculated from surgical biopsies, is shorter in all instances than that calculated from the hepatic surface radioactivity for an as yet unknown reason (see Fig. 2).

TABLE II. RADIOACTIVITY CONTENT OF THE LIVER OF 6 DOGS SACRIFICED 205 TO 302 DAYS AFTER I.M. OR ORAL ADMINISTRATION OF 1.9 TO 2.55 $\mu c = \mu g$ $Co^{60}B_{12}$

DOG NO.	WEIGHT OF ANIMAL IN POUNDS	WEIGHT OF LIVER IN GRAMS	ROUTE AND DOSE OF $Co^{60}B_{12}$ ADMINISTERED		DAYS AFTER ADMINISTRATION OF $Co^{60}B_{12}$	RADIOACTIVITY OF LIVER BIOPSY SPECIMENS IN $m\mu c$ PER 1 GM. TISSUE			TOTAL RADIOACTIVITY OF LIVER			
			TOTAL $\mu c = \mu g$	$\mu c = \mu g$ PER POUND OF WEIGHT		LEFT SIDE	RIGHT SIDE	AVERAGE	IN $m\mu c$	IN $m\mu c$ PER 1 μc $Co^{60}B_{12}$ ADMINISTERED	IN PER CENT OF PEAK VALUE	IN PER CENT OF DOSE OF $Co^{60}B_{12}$ ADMINISTERED
433	17.0	240	2.55	0.15	1	1.70	2.10	1.90	456	179	73.1	17.9
					23	2.60		2.60	624	245	100.0	24.5
			I.M.	I.M.	35	1.80		1.80	432	170	57.5	17.0
					205	0.29	0.28	0.29	70	28	11.4	2.8
435	14.0	162	2.10	0.15	1	3.80	2.10	2.95	478	228	92.3	22.8
					23	3.20		3.20	518	247	100.0	24.7
			I.M.	I.M.	35	2.90		2.90	470	224	90.7	22.4
					205	0.41	0.40	0.41	66	31	12.5	3.1
436	23.0	268	2.3	0.10	7	1.15	1.45	1.30	348	151	66.2	15.1
					23	1.96		1.96	525	228	100.0	22.8
			I.M.	I.M.	35	1.65		1.65	442	192	84.2	19.2
					91	0.90		0.90	241	104	45.6	10.4
					232	0.25	0.31	0.28	75	33	14.5	3.3
437	19.0	212	1.9	0.10	7	1.20		1.20	254	134	77.0	13.4
					23	1.50	1.62	1.56	330	174	100.0	17.4
			I.M.	I.M.	35	1.30		1.30	276	145	83.3	14.5
					91	0.53		0.53	112	59	33.9	5.9
					232	0.16	0.21	0.19	40	21	12.1	2.1
434	15.0	182	2.25	0.15	14	0.35		0.35	64	26	100.0	2.6
					35	0.22		0.22	40	16	61.5	1.6
			orally	orally								
438	21.0	254	2.5	0.12	14	0.29		0.29	74	30	100.0	3.0
					35	0.18		0.18	46	18	60.0	1.8
			orally	orally	91	0.12		0.12	30	12	40.0	1.2

*Content of Radioactive Vitamin B_{12} in the Organs**.—The data obtained by surgical biopsy of the liver immediately before the death of the animals, correlate well with the figures obtained by the homogenization of the entire organ, as shown by Table III. The difference is less than 10 per cent; the calculation of the content of radioactive B_{12} in the liver, on the basis of the weight of the liver and the average content of $Co^{60}B_{12}$ in the biopsy specimens, is permissible.

TABLE III. COMPARISON OF DATA OBTAINED ON THE CONTENT OF $Co^{60}B_{12}$ IN THE LIVER BY SURGICAL LIVER BIOPSIES AND HOMOGENIZATION OF THE ENTIRE ORGAN

DOG NO.*	DAYS AFTER INJECTION OF $Co^{60}B_{12}$	RADIOACTIVITY IN mμc PER 1 GM. TISSUE			
		SURGICAL LIVER BIOPSY			HOMOGENIZED ENTIRE TISSUE
		LEFT SIDE	RIGHT SIDE	AVERAGE	
433	232	0.290	0.280	0.285	0.310
435	232	0.410	0.400	0.405	0.400
436	205	0.250	0.310	0.280	0.300
437	205	0.160	0.210	0.185	0.170

*Dogs 433 and 435 received the dose of 0.15 μc, and Dogs 436 and 437 the dose of 0.1 μc $Co^{60}B_{12}$ per pound of weight, intramuscularly.

TABLE IV. CONTENT OF RADIOACTIVE $Co^{60}B_{12}$ IN THE LIVER AND KIDNEYS OF 6 DOGS SACRIFICED 205 TO 302 DAYS AFTER I.M. ADMINISTRATION OF $Co^{60}B_{12}$

DOG NO.	436	437	433	435	262	263
Weight (in pounds)	23.0	19.0	17.0	14.0	13.75	16.0
Dose of $Co^{60}B_{12}$ administered I.M.:						
in μc = μg per pound weight	0.10	0.10	0.15	0.15	0.60	0.60
in μc = μg per animal	2.30	1.90	2.55	2.10	8.25	9.60
Day after administration of $Co^{60}B_{12}$	232	232	205	205	218	302
Liver:						
Weight in grams	268	212	240	162	197	242
Total radioactivity in mμc	80	35	74	65	161	78
Radioactivity per Gm. tissue						
in mμc	0.30	0.51	0.31	0.40	0.82	0.32
in mμc per 0.1 μc/pound injected	0.30	0.51	0.20	0.27	0.14	0.05
Kidneys:						
Weight in grams						
left	27	24	29	17	12.5	
right	26	23	32	17	12.5	
both	53	47	61	34	25	38.5
Total radioactivity in mμc						
left	10.0	8.6	15.9	6.9	16.1	
right	9.8	8.0	15.9	5.9	14.3	
both	19.8	16.6	31.8	12.8	30.4	26.0
Radioactivity per Gm. tissue						
left (in mμc)	0.37	0.36	0.55	0.41	1.28	
right (in mμc)	0.38	0.35	0.50	0.35	1.14	
average (in mμc)	0.37	0.35	0.52	0.38	1.21	0.67
average (in mμc per 0.1 μc/pound injected)	0.37	0.35	0.35	0.25	0.20	0.11

The contents of radioactive B_{12} in the liver and kidneys of 6 dogs sacrificed 205 to 302 days after parenteral administration of radioactive B_{12} are listed in Table IV and Fig. 3, and that in the visceral organs of 2 dogs (262 and 263)

*The help of Dr. J. M. Cooperman and Dr. A. L. Luhby, as well as that of Mr. D. Teller, who contributed to the assay of the radioactivity content of the visceral organs of 2 of the dogs, is gratefully acknowledged.

in Table V and Fig. 4. At the end of 7 months, in Dog 262, the total content of $Co^{60}B_{12}$ in the main visceral organs (liver, kidneys, pancreas, spleen, and small intestine) totalled only 3.0 per cent of the dose of $Co^{60}B_{12}$ injected 7 months earlier. In Dog 263, it amounted to only 1.4 per cent of the dose injected 10 months earlier.

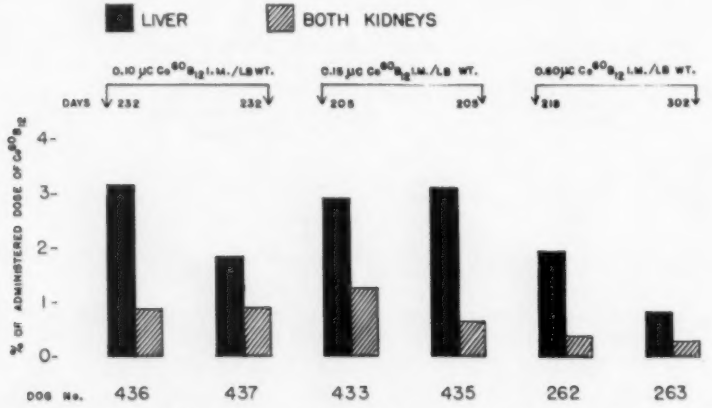

Fig. 3.—Storage of $Co^{60}B_{12}$ in liver and kidneys of 6 dogs 205-302 days after I.M. administration of 0.1 to 0.6 μg $Co^{60}B_{12}$ per pound of weight.

Hepatic radioactivity, when calculated per 1 Gm. tissue and 0.1 μc $Co^{60}B_{12}$ per pound weight injected, was relatively higher when the dose injected was smaller. This is shown by Table IV. The period of time at which these measurements were taken was very similar in all dogs except No. 263 (205 to 232 days).

In 4 dogs sacrificed 205 to 232 days after intramuscular administration of 0.1 to 0.15 μg = μc $Co^{60}B_{12}$ per pound of weight, the content of total B_{12} in the liver was assayed by the *Ochromonas malhamensis* method.* The data listed in Table VI indicate a very advanced state of B_{12} depletion in these dogs at the end of the investigative period. If the B_{12} concentration in the dog's liver, like that in the blood,[20] is similar to that of human beings, then the hepatic B_{12} concentration in our sacrificed dogs was several times lower than normal. This indicates a pronounced B_{12} depletion in the dogs investigated. The $Co^{60}B_{12}$ made only 0.85 to 1.1 per cent of the total B_{12} at the end of the investigative period, and still it could be readily detected, both by recording of the external radioactivity of the liver and by counting of the surgical biopsy specimens.

The concentration of $Co^{60}B_{12}$ in the kidneys, when calculated per gram of organ and 0.1 μg per pound $Co^{60}B_{12}$ injected, shows a similar decreasing pattern with increasing dose as that of the liver (Table IV).

The kidneys show the highest concentration of $Co^{60}B_{12}$, followed by the pancreas, the spleen, and the liver (Table V and Fig. 4). The latter 3 organs

*These assays were performed through the courtesy of Dr. H. Baker, Department of Chemistry, Mount Sinai Hospital, New York, N. Y.

have a rather similar concentration, which amounts to from one-half to two-thirds of that of the kidneys. The concentration of $Co^{60}B_{12}$ in the lungs and small intestine is 16 to 26 per cent, and that of the bile 3 to 7 per cent of the concentration of $Co^{60}B_{12}$ in the liver, per gram of weight. The ratio of concentration of $Co^{60}B_{12}$ in the kidney to that of the liver is in the range of 1.0 to 2.2 when calculated per gram of tissue. The distribution of $Co^{60}B_{12}$ between both kidneys is equal and within the limits of the errors of the method.

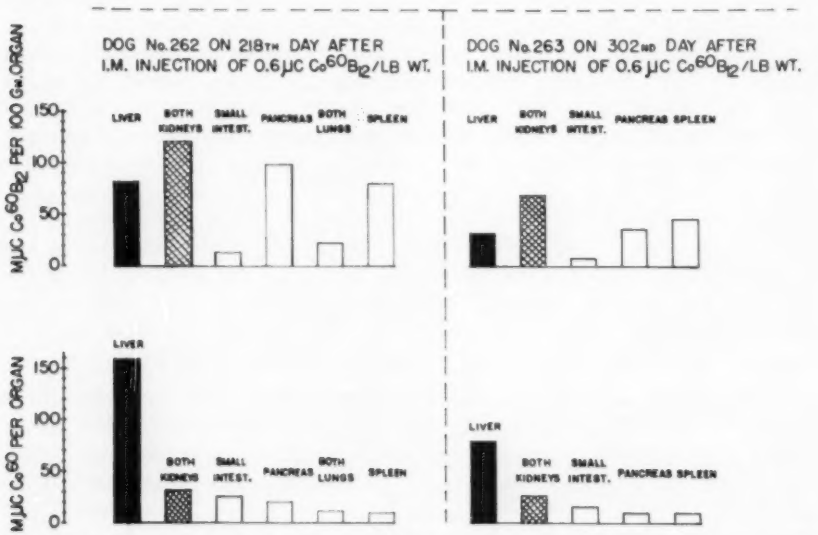

Fig. 4.—Distribution of $Co^{60}B_{12}$ in visceral organs of 2 dogs 218 and 302 days, respectively, after I.M. injection of 0.6 μc $Co^{60}B_{12}$ per pound of weight.

TABLE V. CONTENT OF RADIOACTIVE $Co^{60}B_{12}$ IN THE VISCERAL ORGANS OF 2 DOGS SACRIFICED 7 AND 10 MONTHS AFTER I.M. ADMINISTRATION OF 0.6 μg (μc) $Co^{60}B_{12}$ PER POUND OF WEIGHT

ORGAN	WEIGHT OF ORGAN IN GM.		TOTAL RADIOACTIVITY OF $Co^{60}B_{12}$ IN mμc		RADIOACTIVITY IN mμc OF $Co^{60}B_{12}$ PER GM. OF ORGAN	
	DOG 262* on DAY 218	DOG 263† on DAY 302	DOG 262 on DAY 218	DOG 263 on DAY 302	DOG 262 on DAY 218	DOG 263 on DAY 302
Liver	196.9	242.0	161.0	77.8	0.82	0.32
Kidney (1)	12.6		16.1		1.28	
Kidney (2)	12.5		14.3		1.14	
Kidney (both)	25.1	38.5	30.4	26.0	1.21	0.67
Pancreas	21.1	21.0	20.6	7.3	0.98	0.35
Spleen	12.1	22.5	9.6	10.3	0.79	0.46
Small intes.	189.9	215.7	24.6	15.7	0.13	0.07
Lung (1)	30.3		6.8		0.22	
Lung (2)	22.5		4.5		0.20	
Lung (both)	52.8		11.3		0.21	
Uterus	43.2		6.3		0.15	
Bile	5.2	5.4	0.3	0.07	0.06	0.01

*Weight at start of experiment, 13.75 pounds.
†Weight at start of experiment, 16.0 pounds.

The total content of $Co^{60}B_{12}$ is highest by far in the liver, owing to the greater weight of this organ. In second place, but rather distant, are the kidneys, then the small intestine, pancreas, lungs, spleen, and uterus (Table V and Fig. 4). The total content of $Co^{60}B_{12}$ in the liver is 2.1 to 5.3 times greater than that of both kidneys combined (Fig. 3). The content of $Co^{60}B_{12}$ in all the main visceral organs combined is less than that of the liver alone.

Dog 263 gave birth to a litter of 4 on the two hundred and thirty-second day after intramuscular injection of the tracer. In all 4 puppies, radioactivity was recorded with the scintillation counter over two projections (the anterior and right lateral) of the liver. Hepatic surface radioactivity was 121 counts per minute above background in the first puppy, 97 in the second, 109 in the third, and 113 in the fourth. Since the hepatic radioactivity of the mother amounted to 2,501 c.p.m. at that time, it would appear that some 18 per cent of the hepatic radioactivity of the mother was found in the livers of the 4 puppies. This calculation is very rough, in view of the different geometry of the liver of a puppy as compared to that of an adult dog. In any case, the placental transfer of some portion of the $Co^{60}B_{12}$ from the mother to the offspring certainly resulted in the lowering of the hepatic radioactivity during the last 3 months prior to delivery. Otherwise, the slope of the tracing of the hepatic surface radioactivity, shown in Fig. 1, in Dog 263, would be less pronounced, and the biologic decay of $Co^{60}B_{12}$ in the liver would be slower.

TABLE VI. CONTENT OF $Co^{60}B_{12}$ AND TOTAL VITAMIN B_{12} IN THE LIVERS OF 4 DOGS SACRIFICED 205 TO 232 DAYS AFTER I.M. ADMINISTRATION OF 0.1 TO 0.15 $\mu g = \mu c$ $Co^{60}B_{12}$ PER POUND OF WEIGHT

DOG NO.	436	437	433	435
$Co^{60}B_{12}$ $m\mu g = m\mu c$ per Gm. in liver tissue	0.30	0.51	0.31	0.40
Total B_{12} $m\mu g$ per Gm. in liver tissue	27.00	46.00	35.00	47.00
Ratio $Co^{60}B_{12}$/Total B_{12} in per cent in liver tissue	1.10	1.10	0.88	0.85

DISCUSSION

The pattern of the deposition of radioactive B_{12} in the liver of dogs, following its parenteral administration, is similar to that observed in human beings. The hepatic radioactivity in the dogs during the first weeks after injection increases. This is similar to the hepatic accumulation gradient observed in man.[9-13, 24-26] Both probably represent release of some B_{12} from other sites of the body and its relocation to the liver at this time. The correlation between the amount of radioactivity deposited in the liver and the amount of radioactive $Co^{60}B_{12}$ injected is as evident in dogs as it was in our earlier work on human beings.[9] The 2 dogs to whom $Co^{60}B_{12}$ was administered orally show a very limited absorption of B_{12} in the intestine. This "mucosal block" is similar to the limited intestinal absorption of vitamin B_{12} observed in humans.[27, 28]

The patterns of storage of B_{12} in the liver and kidneys of dogs also appear to be similar to those in man. The very high concentration per gram of wet weight of $Co^{60}B_{12}$ in the kidneys, which is higher than that in the liver of

dogs, was also observed in earlier work of Williganand associates,[21] in which a similar sequence of concentration of B_{12} in the visceral organs of a dog was noted as that described in our study. This was also observed by Brueer[23] in a terminal cancer patient to whom $Co^{56, 57}$-labeled vitamin B_{12} was given by injection.

From each dose of B_{12} injected, a large fraction is stored in the liver. This fraction is relatively higher with the injection of small doses. It is less with larger doses, although the total amount of B_{12} stored in the liver will, necessarily, be greater. This suggests that repeated small parenteral doses may load the liver with B_{12} more efficiently than if the same total amount is given in one large dose. One explanation is that a relatively larger amount of B_{12} is lost in the urine when a large single dose of B_{12} is injected. The loss is less when small amounts are injected repeatedly.[22, 29, 30]

The biologic half-life of radioactive B_{12} in the liver of normal dogs is much shorter than in human beings. It ranges from 6 to 12 weeks if small amounts of $Co^{60}B_{12}$ are injected, and up to 16 weeks with larger doses. Schloesser and Schilling[13] set the average biologic half-life of B_{12} in the liver of man as equivalent to about one year. Although our recent data[31] indicate that the biologic half-life in humans may be shorter than one year, it is still much longer than in dogs.

The short biologic half-life time of hepatic $Co^{60}B_{12}$ in dogs can be demonstrated with various doses of the tracer. It does not depend on the B_{12} deficiency of our dogs, since patients with pernicious anemia who had very low B_{12} blood levels, indicative of severe B_{12} depletion, had a biologic half-life time of $Co^{60}B_{12}$ in the liver much longer than did our dogs.[31] The metabolic turnover of vitamin B_{12} in dogs is apparently faster than in man. This may be due to (a) the lower size of the metabolic pool of B_{12} in the dog, when calculated per unit of weight of the animal, (b) to the generally faster rate of metabolism of the dog as compared to man, or (c) to the greater metabolic needs for vitamin B_{12} in the tissues of the dog than those of humans.

The doses of 0.1 and 0.6 μg of $Co^{60}B_{12}$ per pound of weight which we injected into dogs correspond to a dose of 15 to 100 μg, respectively, in human beings. These relatively large doses of tracer were necessary to obtain sufficiently significant data on the distribution, storage, and discharge of $Co^{60}B_{12}$ in our investigations.

Under the conditions of our investigations, there is a steadily decreasing gradient of decline in radioactivity over the cutaneous projections of the liver, as well as in the surgical liver biopsy specimens. The gradient of decline is much faster in the first weeks of our experiments (i.e., 3 to 6 weeks after I.M. administration of $Co^{60}B_{12}$) than later. The biologic half-life of $Co^{60}B_{12}$ in the liver shows a steady prolongation with the progress of the observations. The shape of these curves with the steeper earlier portion tends to indicate that the curve is not a first order function. It is probable that this phenomenon represents the feed-back of vitamin B_{12} from other organs to the liver at that time. This possibility is supported by a similar shift observed in the first 2 to 3

weeks after parenteral administration of $Co^{60}B_{12}$ to human beings. A similar long initial accumulation gradient was observed in humans.[9-13, 24, 25] The rapid clearing of B_{12} through the kidneys at the initial stage of the observations when the stores of $Co^{60}B_{12}$ were higher may also be partly responsible for this phenomenon. One would also have to consider the possibility of multiple pools of vitamin B_{12} and the interchangeability of the vascular and extravascular B_{12} compartments.

The information on the size of the metabolic pool of vitamin B_{12} in the dog is not available, however. It is also unknown whether there is a single, or several, B_{12} pools in the human and animal body, and in which form B_{12} is deposited and stored in various organs.

The rate of discharge of $Co^{60}B_{12}$ from the liver seems to depend also on the amount of $Co^{60}B_{12}$ injected. The more $Co^{60}B_{12}$ injected, the less steep was the gradient of decrease of hepatic radioactivity and the longer was the biologic half-life time of the hepatic $Co^{60}B_{12}$. Possible explanations for this phenomenon are as follows. (1) The rate of discharge of B_{12} from the liver depends on the demand of the tissues for this vitamin. With a larger dose injected (0.6 μg per pound of weight), which would correspond in a human to about 5 per cent of the total B_{12} pool, this demand would be smaller. (2) The rate of discharge of B_{12} from the liver depends on the amount stored in this organ; with a larger dose, this amount would be relatively larger. (3) There is more feed-back from other tissues to the liver with a larger dose than with a smaller. The first two possibilities are less probable, since the changes in the size of the total pool of B_{12} must be rather small at doses of $Co^{60}B_{12}$ injected. In any event, the causes of this phenomenon are probably different from those which speed up the discharge of $Co^{60}B_{12}$ from the liver in humans when large flushing doses of nonradioactive B_{12} are injected.[32] With less intensive flushing, no pronounced effects on the discharge of radioactivity from the liver were observed, however.[11-13, 33]

SUMMARY AND CONCLUSIONS

Hepatic deposition, storage, and discharge of vitamin B_{12} in the dog, as studied by isotope technique, is similar, grossly, to that in man, although the metabolic turnover of B_{12} in the liver of the dog is much faster. A good correlation was observed between the amount of radioactive B_{12} injected and the hepatic radioactivity, as well as between the surface hepatic radioactivity and that determined by counting of the liver biopsy specimens. Three weeks after I.M. administration of $CO^{60}B_{12}$, at the peak of hepatic accumulation of B_{12}, the total radioactivity of the liver, as measured by surgical biopsy, amounted to 17.4 to 24.7 per cent of the total dose of $Co^{60}B_{12}$ injected. With an oral dose of B_{12}, the biologic half-life was shortest of all observed, and deposition of radioactivity in the liver was much below that observed after a similar dose was administered parenterally.

The biologic half-life of $Co^{60}B_{12}$ in the liver of 8 dogs studied was much shorter than that in human beings, and amounted to 6 to 16 weeks. The

biologic half-life time of radioactive B_{12} in the liver was longer when a larger dose of $Co^{60}B_{12}$ was injected, and shorter when a smaller dose was injected or given orally. The decrease in surface radioactivity over the liver showed a much steeper slope at the onset of the investigative period than subsequently. As the discharge of B_{12} from the liver progressed, the surface radioactivity became relatively higher per unit of $Co^{60}B_{12}$ contained in the liver, for reasons yet to be ascertained. Progressive discharge of radioactive B_{12} from the liver of the dogs caused a decrease of the hepatic radioactivity, as determined by consecutive liver biopsies at the end of the seventh to eighth month to 11.4 to 14.5 per cent of the peak value observed 3 weeks after injection.

In 6 dogs sacrificed 205 to 302 days after parenteral administration of radioactive B_{12}, the concentration of radioactive B_{12} was highest in the kidneys, when calculated per 1 Gm. of organ, next highest in the pancreas, then, in descending order, in the spleen, liver, lungs, and small intestine. The total content of radioactive B_{12} in the organs was highest in the liver, and higher than that of all the main extrahepatic visceral organs combined.

The fraction of $Co^{60}B_{12}$ stored in the liver from each dose of B_{12} injected is relatively higher with the injection of smaller doses. It is less with larger doses, although the total amount of B_{12} stored will necessarily be greater. This indicates that repeated small parenteral doses may load the liver with B_{12} more efficiently than if the same total amount is given in one large dose.

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RADIOACTIVE VITAMIN B_{12} IN THE LIVER

III. Hepatic Storage and Discharge of $Co^{60}B_{12}$ in Pernicious Anemia

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THIS investigation was undertaken for the purpose of collecting information on the kinetics of hepatic storage and discharge of radioactive vitamin B_{12} in patients with pernicious anemia.

It has been ascertained that the greater part of the radioactivity in the liver, following administration of $Co^{60}B_{12}$, is not derived from the Co^{60} detached in this organ from the rest of the B_{12} molecule, but from the radioactive B_{12} stored in the liver.[1] Thus, the radioactivity emanating from the liver and recorded over its surface by means of a scintillation counter[2] truly reflects its content in radioactive B_{12}. This recording method was applied to the measurement of hepatic storage and discharge of vitamin B_{12} in patients with pernicious anemia and in controls.

MATERIAL AND METHOD

Investigations were performed on 8 patients with pernicious anemia and on 10 controls, including 2 with nonpernicious macrocytic anemia from other causes, 1 with iron deficiency anemia, 1 with blood loss anemia, 1 following total gastrectomy, 3 following partial gastrectomy, 1 following hepatitis, and 1 with carcinoma of the stomach. In all, there were 23 periods of observation, 7 of which lasted from 1 to 4 months, 9 from 4 to 8 months, 3 from 8 to 12 months, 3 from 12 to 16 months, and 1 for 29 months.

The patients were studied following parenteral or oral administration of radioactive vitamin B_{12}. Observations were started when the hepatic radioactivity reached a peak and began to decline. This usually occurred on the seventh to the fourteenth day after administration of the tracer (0.5 to 1.0 μg $Co^{60}B_{12}$, containing 0.55 to 1.10 μc Co^{60}).* The counting procedure, started at the peak of hepatic counts, was carried out at various time intervals for the entire observation period, as described in the second paper of this series. Reproducibility of the counts was ascertained by painstakingly placing the patient in a supine position on a hard-topped table and by marking the areas of skin which were to be counted by means of indelible dye or by the intradermal injection of a trace of India ink. In each case, the counted areas were also carefully defined by their relation to the vertical anatomic lines. Without exception, the counts were taken with the same equipment and

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at least 2 hours after ingestion of a meal in order to avoid a shift in the hepatic position caused by a distended stomach. In view of the correction of the hepatic counts by means of the $Co^{60}B_{12}$ standards, any reference in this paper to the decay of hepatic radioactivity refers exclusively to the process of biologic discharge of $Co^{60}B_{12}$ from the liver.

To the best of our knowledge, none of the subjects, during the entire observation period, received vitamin B_{12} or folic acid, either orally or by injection. All patients remained on a mixed diet of their own choosing, without any restrictions.

The average rate of discharge of radioactive $Co^{60}B_{12}$ from the liver was calculated, in per cent per month, in 2 ways. First, from the initial and terminal corrected hepatic surface counts, according to the formula $\frac{(a_1 - a_n) \times 100}{a_1 \times t}$ where a_1 and a_n represent the initial and terminal counts, and t is the time in months; and, second, from all the consecutive corrected hepatic surface counts taken during the entire observation period, according to the formula:

$$\left[\frac{(a_1 - a_2) \times 100}{a_1} + \frac{(a_2 - a_3) \times 100}{a_2} \cdots + \frac{(a_{n-1} - a_n) \times 100}{a_{n-1}}\right] : t$$

where a_1, a_2, a_{n-1}, and a_n represent consecutive corrected hepatic surface counts, and t, the time in months of the entire observation period.

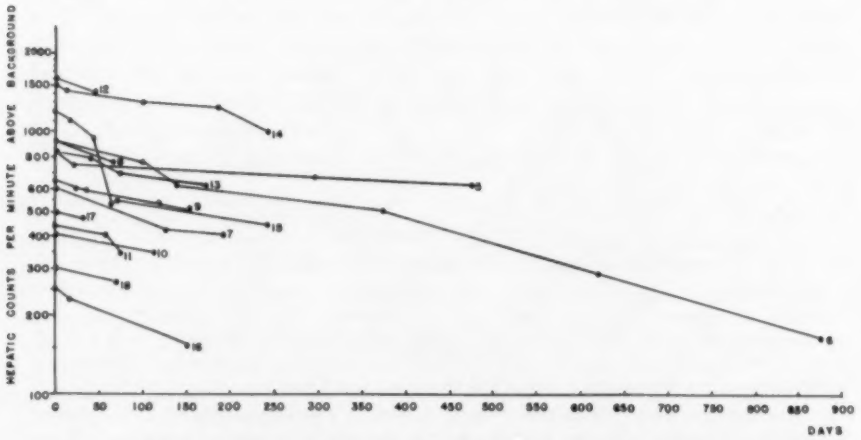

Fig. 1.—Discharge of $Co^{60}B_{12}$ from the liver of 5 patients with pernicious anemia in remission (Cases 5 to 9) and of 9 controls (Cases 10 to 18) measured by hepatic surface radioactivity.

RESULTS

The graphs representing the discharge of radioactive $Co^{60}B_{12}$ from the liver are drawn on semilogarithmic paper in Figs. 1 and 2.

The plot of the discharge of $Co^{60}B_{12}$ from the liver is not exponential, a fact which has also been observed by others.[5] It is, rather, roughly linear, with some erratic changes of the slope of inclination of the composite parts of the plot. These differences in the slopes occur within the same tracing at various times (Cases 6, 7, 11, and 13 to 16 in Fig. 1) and may be pronounced. The rate of discharge of hepatic $Co^{60}B_{12}$, in per cent per month, varies; for instance, in Case 6 from 1.9 to 12.5 per cent at different times; in Case 7 it is in the range of 2.6 to 5.8 per cent; in Case 13, from 2.8 to 11.1 per cent; in

Case 11, 3.7 and 24.9 per cent; in Case 14, 2.0 and 13.1 per cent; and, finally, in Case 15, it varies within the same tracing in the range of 3.9 to 18.2 per cent.

Moreover, the slopes of the tracings of individual patients with pernicious anemia, as well as those of the controls, differ from each other, indicative of the varying rates of discharge of radioactive B_{12} from the liver in various patients (Fig. 1).

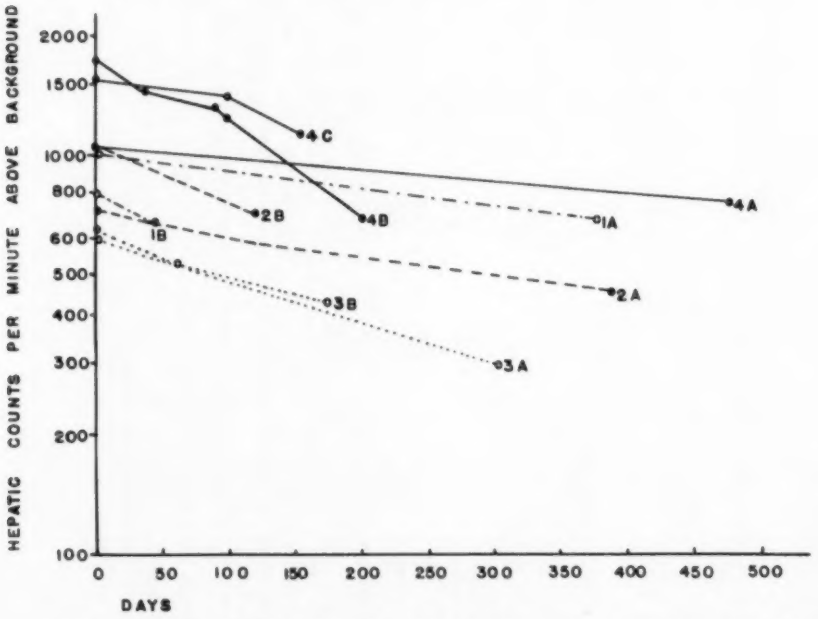

Fig. 2.—Discharge of $Co^{60}B_{12}$ from the liver of 1 patient with nutritional anemia (Case 1) and 3 patients with pernicious anemia in remission (Cases 2 to 4) measured by hepatic surface radioactivity in 9 follow-up studies.

The discharge of radioactivity from the liver of one patient with nutritional macrocytic anemia and of 3 patients with pernicious anemia in remission was repeatedly studied at various observation periods (Fig. 2). The elapsed time between the end of one observation period and the beginning of the other ranged from 1 to 5 months. Observations 1A, 2A, 3A, and 4A were started immediately after the conclusion of intensive parenteral treatment with nonradioactive B_{12}. Observations 1B, 2B, 3B, and 4B were begun 2 to 5 months after the conclusion of observation periods 1A, 2A, 3A, and 4A, and observation 4C was begun one month after conclusion of 4B.

In 3 of 4 cases studied immediately after intensive parenteral treatment with B_{12}, a much slower rate of discharge of radioactivity from the liver was observed (tracings 1A, 2A, and 4A) than at a later date (tracings 1B, 2B, 4B, and 4C). The differences in the slopes of the curves in the same individual at different times and in various individuals are again apparent.

TABLE I. RATE OF DISCHARGE OF $Co^{60}B_{12}$ FROM THE LIVER IN 10 CONTROLS

CASE NO.	PATIENT, SEX, AND AGE	DIAGNOSIS	OBSERVATION PERIOD	DURATION OF OBSERVATION PERIOD IN DAYS	DECREASE OF HEPATIC SURFACE RADIOACTIVITY IN PER CENT			APPROXIMATE BIOLOGIC HALF-LIFE† IN MONTHS
					PER TOTAL PERIOD OF OBSERVATION	PER MONTH*	PER MONTH†	
1	H. S., f., 46	Nutritional macrocytic anemia	a	377	30.2	2.4	2.4	20.8
			b	45	15.9	10.6	10.6	4.7
10	G. LaP., f., 61	After hepatitis	–	101	13.9	4.1	3.8	13.2
11	F. T., m., 67	Ca. stomach	–	72	20.0	8.3	9.0	5.6
12	J. W., m., 29	After subtotal gastrectomy	–	45	4.7	3.1	3.1	16.1
13	L. R., f., 38	After subtotal gastrectomy	–	169	25.4	4.5	4.9	10.2
14	Y. T., m., 46	After subtotal gastrectomy	–	243	32.8	4.1	2.7	18.5
15	J. J., m., 60	After total gastrectomy	–	238	61.7	7.8	11.2	4.5
16	P. M., m., 73	Macrocytic anemia in sprue	–	150	40.0	8.0	8.6	5.8
17	E. N., f., 55	Iron deficiency anemia	–	31	4.2	4.0	4.0	12.5
18	J. H., m., 63	Hypochromic blood loss anemia	–	68	8.4	3.7	3.7	13.3
Range				31-243	4.2-61.7	2.4-10.6	2.4-11.2	4.7-20.8
Mean				139	23.4	5.5	5.8	11.4† 8.6‡

*Calculated from the initial and terminal counts according to the formula: $\frac{(a_1 - a_n) \times 100}{a_1 \times t}$ where a is the initial corrected hepatic surface radioactivity in c.p.m., a_n, the terminal corrected hepatic surface radioactivity, and t is the time in months of the entire observation period.

†Calculated from all consecutive counts of hepatic surface radioactivity extending over the entire observation period according to the formula:

$$\left[\frac{(a_1 - a_2) \times 100}{a_1} + \frac{(a_2 - a_3) \times 100}{a_2} \cdots + \frac{(a_{n-1} - a_n) \times 100}{a_{n-1}}\right] : t$$

where $a_1 \ldots a_{n-1}$, a_n are consecutive corrected hepatic surface counts, and t is the time in months of the entire observation period.

‡Calculated from the mean decrease of hepatic radioactivity per month.†

TABLE II. RATE OF DISCHARGE OF $Co^{58}B_{12}$ FROM THE LIVER IN 8 PATIENTS WITH PERNICIOUS ANEMIA IN REMISSION

CASE NO.	PATIENT, SEX, AND AGE	DIAGNOSIS	OBSERVATION PERIOD	DURATION OF OBSERVATION PERIOD IN DAYS	DECREASE OF HEPATIC SURFACE RADIOACTIVITY IN PER CENT			APPROXIMATE BIOLOGIC HALF-LIFE† IN MONTHS
					PER TOTAL OBSERVATION PERIOD	PER MONTH*	PER MONTH†	
2	J. deW., f., 66	Pernicious anemia in remission	a	350	34.0	2.9	2.9	17.2
			b	123	31.3	7.6	7.6	6.6
3	M. H., f., 65	Pernicious anemia in remission	a	301	52.3	5.2	5.9	8.5
			b	174	26.6	4.6	6.9	7.2
4	P. M., m., 77	Pernicious anemia in remission	a	478	26.7	1.7	1.7	29.4
			b	214	57.8	8.1	10.2	4.9
			c	155	25.6	4.9	4.8	10.4
5	A. L., f., 70	Pernicious anemia in remission	–	485	24.9	1.5	1.7	29.4
6	N. V., f., 75	Pernicious anemia in remission	–	878	81.0	2.8	4.6	10.9
7	V. McD., f., 62	Pernicious anemia in remission	–	191	32.8	5.2	4.7	10.6
8	G. S., m., 44	Pernicious anemia in remission	–	64	17.1	8.0	8.0	6.3
9	M. McC., f., 65	Pernicious anemia in remission	–	149	20.8	4.2	4.4	11.4
Range				64-878	17.1-81.0	1.5-8.1	1.7-10.2	4.9-29.4
Mean				297	35.9	4.7	5.3	12.7† 9.4‡

*Same as in Table I.
†Same as in Table I.
‡Same as in Table I.

In Tables I and II, the data are listed on the range and average values of the rate of discharge of radioactive $Co^{60}B_{12}$ from the liver in 10 controls and 8 patients with pernicious anemia. The mean values as well as the range of decrease in hepatic surface radioactivity, calculated in per cent per month, are grossly similar when calculated by both formulas described above. They are also similar in both groups. The individual variations were wide, however, from case to case, ranging from 1.7 to 11.2 per cent. In 4 cases (1 to 4), marked differences were noted in different observation periods.

There is no evidence that the slopes of hepatic discharge of radioactivity in the control group are less steep than in those with pernicious anemia. The rate of discharge of radioactivity from the liver is even more rapid in some controls than in patients with pernicious anemia in remission.

The slowest rate of discharge of radioactive B_{12} from the liver was noted in 2 patients with pernicious anemia in remission immediately after extensive parenteral treatment with large doses of vitamin B_{12}. The same was true for a patient with nutritional megaloblastic anemia due to dietary B_{12} deficiency. The vitamin B_{12} level in the blood in these 3 instances, assayed at the end of the observation periods, was very low, 40 to 60 $\mu\mu$g per 1 ml. of serum. In spite of a pronounced vitamin B_{12} deficiency, the rate of discharge of radioactive B_{12} from the liver was still exceedingly slow.

Since the plot of the discharge of $Co^{60}B_{12}$ from the liver is linear rather than exponential, it is justified to calculate the approximate biologic half-life of $Co^{60}B_{12}$ in the liver from the rate of discharge of hepatic $Co^{60}B_{12}$ calculated according to the second formula, i.e., from the consecutive hepatic surface counts taken over the entire observation period. Biologic half-life of $Co^{60}B_{12}$ in the liver, in months, = 50 per cent: mean rate of discharge of $Co^{60}B_{12}$ from the liver in per cent per month.

These calculations are listed in Tables I and II. The values are to be considered only approximative because of the wide range of variability of the rates of discharge of $Co^{60}B_{12}$ from the liver in the same individual at various times.

The average biologic half-life in our observations was 11.4 months in controls, and 12.7 months in patients with pernicious anemia in remission. As shown in Tables I and II, it varied widely from case to case, however, in a wide range of from 4.7 to 29.4 months. It also changed markedly in the same individual at different periods of observation, with extreme values exceeding, for a short time, the range of averages of the biologic half-lives listed in Tables I and II.

DISCUSSION

In animal investigations,[3, 4] as well as in studies in man,[2, 5] the inference has been drawn that vitamin B_{12} remains in the liver for many months following parenteral or oral administration. Other workers have since reported similar findings.[6-9] The average biologic half-life of $Co^{60}B_{12}$ in the liver, in both controls and patients with pernicious anemia in remission, is one year

in our series of 23 observations. This corresponds exactly to the average figure reported by Schloesser and Schilling, obtained by using the same technique.[8] The biologic half-life of B_{12} in the liver varies widely, however, from case to case, and also changes in the same individual at various stages during the period of observation.

The exceedingly slow rate of turnover of radioactive B_{12} in the liver is very striking. Comparison of these data with those obtained on normal dogs[10] whose stores of vitamin B_{12} have been depleted indicates a much longer period of hepatic storage of radioactive B_{12} in human beings than in dogs, where it amounts to only 6 to 16 weeks.

There was no evidence in our findings that the rate of discharge of radioactive B_{12} from the liver in pernicious anemia in remission was different from that in control subjects (subtotal or total gastrectomies, cancer of the stomach, other anemias, or posthepatitis). This is in agreement with the findings of Mollin and associates[11] which indicate that patients with pernicious anemia do not show a greater retention of injected radioactive vitamin B_{12} than do normal individuals. The same is apparently true for the hepatic storage of radioactive vitamin B_{12}.

It is obvious that it was impossible to collect similar data on patients with pernicious anemia at acute onset, or in relapse, who could not be left without treatment for such a long observation period.

It is to be expected that the rate of discharge of B_{12} from the liver will be slower in patients saturated with vitamin B_{12} than in those with advanced B_{12} deficiency. That this may be so is perhaps indicated by the fact that our patients, after an extensive course of parenteral B_{12} treatment, showed a slower rate of discharge of radioactive B_{12} from the liver than at other times.

Individual differences in the slope of hepatic radioactivity tracings may depend on the state of saturation or depletion, as the case may be, of the organism's store of vitamin B_{12}. Because of the limited scope of our observations, however, no inferences are warranted as to the extent of these correlations.

SUMMARY AND CONCLUSIONS

In 18 individuals, including 8 patients with pernicious anemia in remission, the rate of discharge of radioactive B_{12} from the liver was studied by recording the radioactivity on the surface of the liver following oral or parenteral administration of a tracer dose of $Co^{60}B_{12}$ by means of a scintillation probe. The investigation was conducted through 23 observation periods which extended, in 16 instances, from 4 to 29 months each in length.

The monthly decrease in hepatic radioactivity was from 1.7 to 11.2 per cent with the average value at 5.5 per cent. This corresponds to a biologic half-life of $Co^{60}B_{12}$ in the liver of an average of 12.1 months, the range extending from 4.7 to 29.4 months.

The rate of discharge of $Co^{60}B_{12}$ from the liver varies within the same observation period at different times. In 3 patients with pernicious anemia

and in 1 with nutritional macrocytic anemia, the rate of discharge of $Co^{60}B_{12}$ from the liver also varied at different observation periods, with the hepatic half-life ranging from 4 to 16 months in the same individual.

No evidence was obtained that the rate of discharge of $Co^{60}B_{12}$ from the liver was different in patients with pernicious anemia in remission from that of the controls studied.

In 3 cases of pernicious anemia, the rate of discharge of $Co^{60}B_{12}$ from the liver was exceedingly slow following the saturation of the B_{12} stores by extensive parenteral treatment with vitamin B_{12} and, also, much slower than at other times.

The hepatic storage of vitamin B_{12} in human beings is very prolonged, but the biologic half-life of B_{12} in the liver may vary over a wide range.

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THE EFFECT OF MECAMYLAMINE ON CORONARY FLOW, CARDIAC WORK, AND CARDIAC EFFICIENCY IN NORMOTENSIVE DOGS

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INVESTIGATION of the ganglion blocking drugs, hexamethonium and pentolinium, has indicated that their administration is accompanied by a decrease in the central venous pressure,[1-5] cardiac output,[1, 2, 4, 6-9] cardiac work,[4, 6] and, at least with hexamethonium, a decrease in coronary blood flow.[4] Although there are some exceptions to this general pattern[10-12] the discrepancy between the studies seems to be produced by the fact that when blood pressure falls greatly the cardiac output falls but when it falls only slightly the cardiac output tends to stay the same or even rise slightly.[9] Some properties of mecamylamine which set it apart from preceding agents, notably its chemical structure (3-methylaminoisocamphane hydrochloride),[13] its relatively complete absorption when administered by mouth,[13, 14] and its passage across cell boundaries so that it is deposited intracellularly in the body,[14] as well as its myocardial depressant properties,[15] have indicated that it might be worth while to investigate its circulatory effects to see if they also differ from the foregoing ganglion blocking drugs.

MATERIALS AND METHODS

This study was done on mongrel dogs weighing from 17 to 24 kilograms. The basic method used in this study has been described previously from this laboratory.[5] Anesthesia was secured by intramuscular administration of 3 mg. per kilogram of weight of morphine sulfate followed in 1 hour by 12 mg. per kilogram of sodium pentobarbital intravenously. Cardiac catheters were maneuvered under fluoroscopy into the pulmonary artery, the coronary sinus, and the right atrium. Cardiac output was determined by the Fick principle and coronary blood flow by the nitrous oxide saturation method before and an average of 20 minutes after the intravenous administration of 0.5 to 1.0 mg. per kilogram of mecamylamine. Mean blood pressures were determined by electrical integration of the pressure curve emitted from the Statham strain gauges with the Sanborn Polyviso.

RESULTS

The results of the study are summarized in Table I. Following within one to two minutes after the intravenous administration of mecamylamine there was an increase in cardiac rate which was maintained throughout the second cardiac

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output and coronary blood flow determination (+27 per cent, $P<0.01$) and until the end of the 45- to 60-minute period of observation. The mean blood pressure fell and during the experimental periods was 14 per cent lower in the femoral artery ($P<0.001$), 42 per cent less in the pulmonary artery ($P<0.001$), and 1.5 mm. Hg less in the right atrium ($P<0.01$). The decrease in pressure in the pulmonary artery occurred in the first one to two minutes after administration of the drug and tended to rise slowly thereafter. Femoral arterial pressure fell more gradually over several minutes, but both pressures remained quite stable throughout the period of second determinations of cardiac output and coronary blood flow.

TABLE I. SUMMARY OF EFFECTS OF MECAMYLAMINE GIVEN INTRAVENOUSLY

DETERMINATION	BEFORE	AFTER	PER CENT CHANGE	P VALUE
Cardiac rate	98 ± 18	124 ± 26	+27	<0.01
Mean arterial blood pressure (mm. Hg)	106 ± 14	91 ± 12	−14	<0.001
Mean pulmonary arterial blood pressure (mm. Hg)	12 ± 4	7 ± 2	−42	<0.001
Right atrial mean pressure (mm. Hg)	0.3 ± 1.8	−1.2 ± 1.7	−500	<0.01
Arteriovenous O_2 difference (vol./100 ml.)	3.2 ± 0.5	4.6 ± 1.2	+44	<0.05
Arterial-coronary sinus O_2 difference (vol./100 ml.)	10.0 ± 1.4	12.4 ± 1.8	+24	<0.001
Coronary sinus blood O_2 content (vol./100 ml.)	6.5 ± 2.8	3.1 ± 1.2	−52	<0.001
Cardiac output (liters per minute)	3.1 ± 1.1	2.2 ± 0.7	−29	<0.01
Coronary blood flow (ml./100 Gm./min.)	104 ± 24	70 ± 18	−34	<0.01
Cardiac O_2 consumption (ml./100 Gm./min.)	10.4 ± 2.5	8.6 ± 2.1	−17	<0.05

A moderate (17 per cent, $P<0.05$) increase in minute volume of respiration occurred in spite of the fact that oxygen consumption and carbon dioxide excretion did not change and consequently there was a significant decrease in oxygen consumption and carbon dioxide excretion per liter of ventilation (−21 per cent, $P<0.001$). The fact that the body respiratory quotient was not changed indicated that the animals had reached a steady state by the time of the second study.

From the control to the experimental study there was a slight fall in the arterial oxygen content (−7 per cent, $P<0.001$), and a greater decrease in the mixed venous oxygen content (18 per cent, $P<0.01$) with widening of the arteriovenous oxygen difference (+44 per cent, $P<0.05$). There was similar widening of the mixed venous-arterial carbon dioxide content (+48 per cent, $P<0.01$). The coronary arteriovenous oxygen difference increased (+24 per cent, $P<0.01$) chiefly because of the fall in coronary sinus oxygen content (−3.4 volumes per 100 ml., $P<0.001$). On the other hand, the coronary sinus carbon dioxide content did not change significantly, but, since the arterial carbon dioxide content fell, the coronary sinus-arterial carbon dioxide difference increased by 31 per cent ($P<0.001$). Decreases occurred in both the arterial hemoglobin and the arterial hematocrit (−5 per cent, $P<0.01$). Arterial pH did not change significantly, although there was a trend upward (+0.03, $P<0.1$).

The cardiac output decreased an average of 29 per cent ($P<0.01$). Since there was an increase in the cardiac rate at the same time, the stroke volume

fell even more markedly (–41 per cent, $P<0.001$). The left ventricular work decreased an average of 38 per cent ($P<0.01$), while right ventricular work decreased 60 per cent ($P<0.01$), reflecting the more marked decrease in pulmonary arterial pressure. The calculated peripheral and pulmonary vascular resistances were variable, but the total peripheral resistance showed a trend to increase (+27 per cent, $P<0.1$).

Coronary blood flow decreased an average of 34 per cent ($P<0.01$). Oxygen consumption per 100 Gm. of left ventricular myocardium decreased (–17 per cent, $P<0.05$), but the carbon dioxide liberation per 100 Gm. of left ventricular myocardium was so variable that the 14 per cent decrease was not significant. The cardiac respiratory quotient did not change. The coronary vascular resistance increased significantly (+27 per cent, $P<0.02$), and the index of efficiency (determined by dividing the left ventricular work by the oxygen consumption per minute of 100 Gm. of left ventricular myocardium) decreased significantly (–27 per cent, $P<0.02$).

DISCUSSION

Previous studies have shown a decrease in cardiac output of dogs[5, 8] and man receiving hexamethonium[1-3] and of man receiving pentolinium.[6, 7] With both drugs there was a decrease in the central venous filling pressure which presumably accounted for the decreased output.[6-9] In some cases, particularly when the fall in blood pressure was not great, cardiac output remained the same or rose slightly.[3, 6, 10-12] Studies of the response of the isolated heart[16] and the heart-lung preparation[17] have shown that low doses of quaternary ammonium salts such as those used in these studies have no effect and high doses (30 mg. per kilogram) have a positive inotropic action. These observations, coupled with the decrease in central venous filling pressure in the present study, furnish no proof that the myocardium itself is depressed. Experiments with strain gauge arches applied to the heart directly, however, have shown decreased force of contraction after administration of hexamethonium. The authors attributed this decrease to the lowered arterial pressure, decreased autonomic activity or direct myocardial action.[18]

Mecamylamine produced decreased rate and amplitude of contraction of isolated perfused cat, rabbit, and guinea pig hearts.[15] That this may not apply in the intact dog with the dose used in this study is emphasized by the marked increase in cardiac rate and decrease in filling pressure, indicating again that, under the conditions of this experiment, myocardial depression probably was not responsible for the decrease in output. A similar discrepancy between in vitro and in vivo studies of myocardial metabolism has been described for quinidine in which oxygen consumption was decreased in isolated cardiac muscle strips but increased in the heart of the intact dog.[19]

The decrease in blood pressure in these experiments, as in preceding experiments with hexamethonium[5, 8] and pentolinium,[6, 7] was not due to a decrease in peripheral resistance but rather to the decrease in cardiac output. In this study the trend was for peripheral resistance to rise since it averaged +27 per cent but

this was not statistically significant ($P<0.1$). One additional study (data at the bottom of Table I) was excluded from this series because, although satisfactory in every other way, a gas mixture containing carbon dioxide inadvertently was used for determining coronary blood flow. If the data concerning peripheral resistance from this study are included with the rest, the increase in peripheral resistance becomes significant ($P<0.05$). The pattern of peripheral resistance change in this study is similar to that described by Grob and associates,[9] in that if the cardiac output rises or does not decrease greatly the peripheral resistance falls but when the output decreases significantly peripheral resistance rises. Trapold[20] confirmed and extended this thesis by demonstrating a decrease in resistance of perfused mesenteric vessels on administration of ganglion blocking drugs. When the perfusing pressure and hence the rate of perfusion was decreased greatly, peripheral resistance rose sharply. This was construed to mean that when flow fell too much the closure of capillary beds caused an increase in resistance. Any teleologic explanation for the increased peripheral resistance which involves maintenance of visceral blood flow after administration of ganglion blocking drugs must reckon with the identical (+27 per cent) but more consistent and significant ($P<0.02$) rise in coronary vascular resistance and the reduced coronary blood flow.

A decrease in cardiac efficiency for accomplishment of external work such as has been demonstrated both in this study and in the preceding study of hexamethonium,[5] must be interpreted in the light of the considerable increase of cardiac rate. Preceding work in the heart-lung preparation,[21] isolated metabolically supported heart,[22] and in the intact animal[23] have shown a considerable reduction in myocardial efficiency during increased cardiac rates. Furthermore, in the isolated metabolically supported heart efficiency tends to fall as the cardiac output falls and the amount of external work done decreases.[22]

SUMMARY

In 10 normotensive dogs studies were made before and an average of 20 minutes after intravenous administration of 0.5 to 1 mg. per kilogram of mecamylamine. There were significant decreases in systemic, pulmonary arterial, and right atrial mean blood pressures accompanied by an increase in cardiac rate. Body oxygen consumption and carbon dioxide liberation did not change and the respiratory quotient was unchanged. There was significant widening of the arteriovenous oxygen and carbon dioxide differences, however, and a fall in cardiac output. Cardiac work decreased as did coronary blood flow, myocardial oxygen consumption, and calculated cardiac efficiency. Coronary vascular resistance increased. These observations are compared with those of the preceding ganglion blocking agents, hexamethonium and pentolinium, and in so far as data are available found to be similar.

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THE USE OF A LYOPHILIZED HUMAN PLASMA STANDARDIZED FOR BLOOD COAGULATION FACTORS IN THE COAGULASE AND FIBRINOLYTIC TESTS

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THERE exists a need in diagnostic laboratories for simple procedures for the differentiation of pathogenic from nonpathogenic staphylococci and streptococci. In current use are several criteria which indicate that there is a significant correlation between in vitro biochemical activity and the incidence of pathogenicity. The production of coagulase by some strains of staphylococci and the fibrinolytic activity of β-hemolytic streptococci have received increased acceptance in each case as the best single criterion of potential pathogenicity. While the invalidity of any concept of pathogenicity based solely on a single biochemical property is generally recognized, the high frequency of association of the two above-indicated properties with pathogenicity attests to their real value as diagnostic criteria.

Although the role of plasma in these reactions is firmly established, the nature of the reactions and the influence of various plasmas on the detection of these reactions has been controversial for many years.[1-8] Much of the conflicting data is undoubtedly due to the variable concentration of accessory blood-clotting factors in different plasmas, and to the rapid deterioration of these factors in freshly drawn plasma. It has been established that the susceptibility of plasma to staphylocoagulase varies with the animal species used[9] and the presence or absence in such plasma of an activator.[5] Although the relationship of this activator to the various factors concerned with blood coagulation is as yet not fully understood, there is evidence that this activator, called the coagulase-reacting factor by Tager,[5] is similar to, part of, or identical with prothrombin. Current theories concerning the mechanism of fibrinolysis, proposed by Astrup,[10] Müllertz,[11] and by Kline and Fishman,[12] disagree as to the factor in plasma which combines with streptokinase to activate the conversion of plasminogen to plasmin. However, they all agree that this conversion occurs optimally in human as opposed to animal plasmas.

Only 75 per cent of fresh, "normal" human plasmas are suitable for the fibrinolytic test because antistreptokinase or inhibitors are present in the others.

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Since freshly drawn human plasma becomes less suitable for both tests in several hours unless immediately frozen or lyophilized, it was felt that a readily available human plasma standardized for blood-clotting factors could provide an ideal substrate for determining coagulase and fibrinolytic activities. In view of this, and inasmuch as there has been available for some time a lyophilized human plasma preparation that is standardized for prothrombin activity,[*13, 14] we became interested in the evaluation of this material in the coagulase and fibrinolytic tests.

The results of these evaluations form the basis of this report.

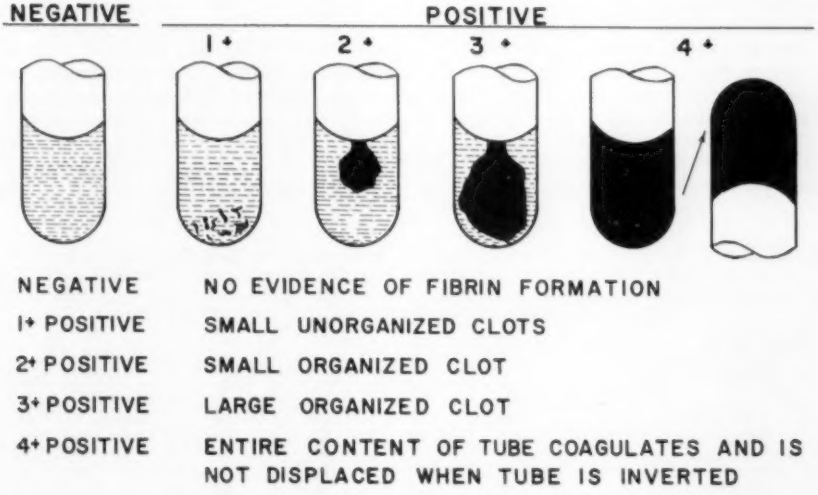

Fig. 1.—Method of scoring coagulase test reactions.

MATERIALS AND METHODS

Plasma.—Three types of plasma were compared in these experiments: (1) a commercially available lyophilized human plasma,* normally used as a prothrombin time control; (2) a commercially available desiccated rabbit plasma†; and fresh oxalated human plasma (potassium oxalated, 1.0 mg. per milliliter of blood) from normal donors.

The lyophilized human plasma was reconstituted by adding 0.5 ml. of distilled water (pH 6.8 to 7.0) to the 0.5 ml. vials. The contents of several vials were pooled and subsequent dilutions made with distilled water, as will be indicated in the text. The desiccated rabbit plasma was reconstituted by dissolving the contents of one ampul (100 mg.) in 3.0 ml. of distilled water. The reconstituted desiccated rabbit plasma and the fresh human plasma were used without further dilution in the coagulase tests.

Organisms.—Forty-three strains of *Staphylococcus pyogenes* and 19 strains of β-hemolytic streptococci were used. Most of these strains were fresh isolates from clinical specimens, and several were stock laboratory strains assembled from various sources.

*Diagnostic Plasma, Warner-Chilcott Laboratories, Morris Plains, N. J.
†Bacto-Coagulase Plasma Difco Laboratories, Detroit, Mich.

Prior to testing, the organisms were grown in brain or beef heart infusion broth (Difco) for 18 to 24 hours at 37° C. For the streptococcal cultures, the medium was fortified with 2 to 3 drops of sterile rabbit serum.

Coagulase Test.—The test tube method was used. In 12 by 75 mm. test tubes 0.5 ml. of an 18- to 24-hour broth culture of the test organism was added to 0.5 ml. of plasma. The tubes were shaken and incubated in a water bath at 37° C. Observations for clotting of the tube contents were made at periods of ½, 1, 2, and 3 hours. The nature of the clot formed and our method of scoring is illustrated in Fig. 1. Any degree of clotting, however slight, occurring within this period was taken as positive evidence of coagulase production. Plasma controls and other appropriate controls were included in each test.

Fibrinolytic Test.—The tube method was used. In 12 by 75 mm. test tubes, 0.5 ml. of an 18- to 24-hour broth culture of the test organism and 0.25 ml. of a 0.25 per cent calcium chloride solution in 0.85 per cent sodium chloride were added to 1.0 ml. of the proper dilution of the test plasma. The plasma, culture, and calcium chloride solutions were immediately mixed with sterile wooden applicator sticks to ensure proper mixing and rapid and complete clot formation. The tubes were incubated in a water bath at 37° C. for approximately 10 minutes, and examined for clot formation. Only those tubes showing complete coagulation were used in the test. The tubes were then reincubated and observed frequently for evidence of lysis over a 24-hour period. Lysis to any degree during this test period was regarded as a positive test for streptokinase production as long as there was no evidence of lysis in the uninoculated controls. In most instances the positive tests showed complete lysis of the clot.

TABLE I. EFFECTS OF SODIUM CHLORIDE CONCENTRATION ON THE COAGULATION OF RECONSTITUTED LYOPHILIZED HUMAN PLASMA IN THE COAGULASE TEST

PER CENT NaCl IN RECONSTITUTED PLASMA	STAPHYLOCOCCUS PYOGENES COAGULASE-POSITIVE STRAINS CLOT FORMATION												
		M-3 (WEAK)				M-4 (INTERMEDIATE)				M-74 (STRONG)			
	(HOURS)	½	1	2	3	½	1	2	3	½	1	2	3
0.28		2+	2+	2+	2+	3+	3+	3+	3+	4+	4+	4+	4+
0.43		1+	1+	2+	2+	2+	2+	3+	3+	4+	4+	4+	4+
0.85		–	–	1+	1+	2+	2+	3+	3+	4+	4+	4+	4+
0.99		–	–	1+	1+	1+	1+	3+	3+	4+	4+	4+	4+
1.00		–	–	–	–	1+	1+	3+	3+	4+	4+	4+	4+
1.13		–	–	–	–	–	1+	3+	3+	4+	4+	4+	4+
1.30		–	–	–	–	–	–	2+	2+	4+	4+	4+	4+
1.70		–	–	–	–	–	–	2+	2+	3+	3+	4+	4+
2.60		–	–	–	–	–	–	2+	2+	3+	3+	4+	4+
3.40		–	–	–	–	–	–	1+	1+	2+	2+	4+	4+
4.20		–	–	–	–	–	–	–	1+	1+	1+	4+	4+

A minus sign indicates no evidence of fibrin formation. Positive reactions are scored as indicated in Fig. 1.

RESULTS

Effect of Salt Concentration in Coagulase Detection.—Lyophilized human plasma was reconstituted to contain sodium chloride concentrations varying from 0.28 to 4.20 per cent. The effects of these salt concentrations on clot formation in the coagulase test were investigated using weak, intermediate, and strong coagulase-producing strains of *Staphylococcus pyogenes.* Sodium chloride concentrations within the range of 0.28 to 1.3 per cent appear to have little effect in the clotting reaction of strong coagulase producers. Concentrations greater than 1.3 per cent delayed the positive reaction for 1 to 2 hours with such strong strains. With the less reactive strains, however, sodium chloride concentrations

above 0.28 per cent progressively delayed or inhibited clot formation. A typical protocol illustrating the influence of salt concentration on coagulase production with lyophilized human plasma is shown in Table I.

Comparison of Various Plasmas in Coagulase Detection.—Forty-three strains of *Staphylococcus pyogenes* were used to evaluate the efficacy of lyophilized human plasma, desiccated rabbit plasma, and fresh oxalated human plasma for the detection of coagulase production. Several lots of the commercially available plasmas were evaluated. The lyophilized human plasma was reconstituted and diluted 1:3 in distilled water. The reconstituted desiccated rabbit plasma and the fresh human plasmas were used undiluted. The data are presented in summary in Table II. Thirty of these strains were positive for coagulase production with all 3 plasmas used. One strain, found negative for coagulase production with fresh human plasma, was weakly positive with the lyophilized human plasma and the desiccated rabbit plasma. One additional strain was positive with the desiccated rabbit plasma and negative with both human plasmas. The nature and type of clot formed with the different plasmas also varied. In most cases, the clots formed with the human plasmas were smaller, and, in several cases, formed later than those seen with the desiccated rabbit plasma.

TABLE II. COMPARISON OF THE COAGULASE REACTIONS OF STAPHYLOCOCCUS PYOGENES WITH FRESH HUMAN PLASMA, LYOPHILIZED HUMAN PLASMA, AND DESICCATED RABBIT PLASMA

PLASMA	NO. OF STRAINS TESTED	COAGULASE REACTION			
		1 HOUR		3 HOURS	
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
Fresh human	43	30	13	30	13
Lyophilized human (Diagnostic Plasma)					
Lot A	43	30	13	31	12
Lot B	43	31	12	31	12
Lot C	43	31	12	31	12
Lot D	43	31	12	31	12
Desiccated rabbit (Bacto-Coagulase)	43	32	11	32	11

Comparison of Lyophilized and Fresh Human Plasma in Fibrinolytic Test.—The fibrinolytic activity of 19 strains of β-hemolytic streptococci was compared, using several lots of lyophilized human plasma and fresh plasma. Both types of plasma were tested at 1:5 dilutions in 0.85 per cent sodium chloride solutions. Twelve of these strains gave a consistent positive reaction with the various lots of lyophilized human plasma. Two of these positive strains failed to lyse one of the oxalated fresh human plasmas. Seven other strains failed to lyse either plasma and were considered negative for streptokinase production. One additional specimen of oxalated human plasma, kept at 5° C. for 18 hours prior to testing, was partially lysed by the active streptokinase-producing strains, but failed to lyse in the presence of less active streptokinase producers. Spontaneous lysis occurred within the 24-hour test period with this sample of plasma.

The results obtained with 4 representative lots of lyophilized human plasma and fresh oxalated human plasma are shown in Table III.

TABLE III. COMPARISON OF THE FIBRINOLYTIC ACTIVITY OF β-HEMOLYTIC STREPTOCOCCI WITH FRESH HUMAN AND LYOPHILIZED HUMAN PLASMA SUBSTRATES

PLASMA	NO. OF STRAINS TESTED	FIBRINOLYTIC ACTIVITY	
		NO. POSITIVE	NO. NEGATIVE
Oxalated human (1)	19	12	7
Lyophilized human (Lot E)	19	12	7
Oxalated human (2)	19	10	9
Lyophilized human (Lot F)	19	12	7
Oxalated human (3)	19	12	7
Lyophilized human (Lot G)	19	12	7
Oxalated human (4)	19	11	8
Lyophilized human (Lot H)	19	12	7

DISCUSSION

Fresh pooled human plasma is considered by many investigators to be the most reliable substrate for the coagulase test. From the results of this study, confirmed by the data reported by Boyd,[15] Waller,[16] and Ness,[17] it is apparent that lyophilized human plasma standardized for blood-clotting factors is as satisfactory as fresh human plasma in detecting coagulase production. In some instances the clots produced by the lyophilized plasma were small, as indicated by 1+ and 2+ reactions. Such reactions, however, are positive responses and should not be overlooked. Any degree of fibrin formation is regarded as a positive reaction.[18]

Because of the existence of species differences in coagulase activators and strain differences in coagulase production,[9] the use of nonhuman plasma in the determination of potential pathogenicity to human beings is of questionable validity.

The optimal sodium chloride concentration in the reconstituted lyophilized plasma was obtained by diluting the lyophilized material 1:3 in distilled water resulting in 0.28 per cent sodium chloride concentration. From the data presented in Table I, it is apparent that higher concentrations of sodium chloride delay or completely inhibit clot formation. In view of this, cultures grown in media containing high salt concentrations should not be used as inocula for the coagulase test. The inhibitory effects of increased sodium chloride concentration were most evident when weak coagulase-producing strains were used. The ionic strength of the final reaction mixture, as determined by conductivity tests, was slightly less than that of physiologic saline.

Plasma of human origin is the optimal substrate for the determination of fibrinolytic activity, since the production of plasmin (fibrinolysin) is dependent on the reaction of streptokinase with a factor found in human plasma.[10-12] However, several workers have shown that approximately 25 per cent of all normal human plasmas are refractory to lysis, and that clots produced with plasma obtained from the blood of patients recovered from hemolytic streptococcal diseases are often totally resistant to liquefaction by active cultures.[6, 19]

The results of this study indicate that a lyophilized human plasma, standardized for blood-clotting factors and coagulase-reacting ability, gave consistently uniform results in the fibrinolytic test regardless of the lot used, while freshly

drawn human plasma gave variable results. One specimen of oxalated human plasma, allowed to remain at 5° C. for 18 hours, failed to lyse in the presence of some weak streptokinase-producing strains. In addition, this specimen lysed spontaneously within the 24-hour test period. The value of a lyophilized human plasma, which is nonrefractory to fibrinolysis, readily available, and convenient to use, is thus apparent.

While fibrinolysis has been used mostly for the detection of potentially pathogenic streptococci, there is some evidence that this same property is manifested by approximately 10 per cent of all coagulase-positive staphylococci.[29] It is evident that this phenomenon warrants investigation, and we suggest that lyophilized human plasma can be used for the detection of staphylokinase production using the procedure outlined for the detection of streptokinase.

It must be emphasized that one of the chief obstacles encountered in the performance of the fibrinolytic test is failure to get complete clotting of the test mixture. This was found to be due to improper mixing of the culture, coagulant, and plasma. This difficulty can be easily overcome and complete clots consistently obtained in less than 10 minutes by mixing these ingredients with sterile wooden applicator sticks.

SUMMARY

1. A lyophilized human plasma, standardized for blood-clotting factors in a dilution of 1:3 in distilled water, gave essentially the same results as freshly drawn human plasma in the coagulase test.

2. Optimal results were obtained when the lyophilized plasma was reconstituted so as to contain a sodium chloride concentration of 0.28 per cent, the ionic strength of the final reaction mixture being slightly less than that of physiologic saline. Higher salt concentrations delayed or inhibited clot formation.

3. A chart illustrating the types of clot formation in the coagulase tests and the influence and significance of the various plasmas on such clot formation is presented.

4. The lyophilized plasma, when reconstituted in distilled water and diluted 1:5 in physiologic saline, gave consistently more uniform results in the fibrinolytic test than did unselected fresh human plasma. All lots of the lyophilized plasma tested were nonrefractory to fibrinolysis.

5. The possible use of this lyophilized plasma in the detection and study of staphylokinase-producing *Staphylococcus pyogenes* strains is indicated.

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THE EFFECT OF ETHYLENEDIAMINETETRAACETIC ACID (EDTA) AND ASCORBIC ACID ON TETRACYCLINE BLOOD SERUM CONCENTRATIONS

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CHELATION is the formation of a compound between a metallic ion and an organic molecule having two groups spatially arranged so as to form a ring structure with the metal.[1] The chelate is so stable that the metal is effectively sequestered and consequently is unavailable for its usual reactions.

Ethylenediaminetetraacetic acid (EDTA) is probably the most potent chelating agent studied to date. Recently, it has been shown that citric acid in an encapsulated mixture with tetracycline hydrochloride produced higher blood serum concentrations of the antibiotic than resulted from the administration of an equivalent amount of the antibiotic alone.[2] In view of the suggestion that this effect may be related to the ability of citrate to form complexes with calcium,[3] it seemed interesting to test this hypothesis by determining whether the same or similar elevations in serum tetracycline concentrations could be achieved by the simultaneous oral administration of EDTA, which is known to effectively bind calcium. Since it had also been reported that ingestion of ascorbic acid also resulted in enhanced tetracycline serum levels,[4] it was decided to include this agent in the study for comparative purposes.

MATERIALS AND METHODS

Medication was administered in gelatin capsules to each of 6 subjects at 11:00 A.M., 2.5 hours after breakfast. Clotted blood specimens were taken immediately prior to administration of medication and again at 2:00 P.M., 2 hours after lunch and 3 hours after medication. All sets of paired sera were assayed for tetracycline activity by an agar diffusion microbiologic method.[5]

Eight different drug regimens were used in a crossover type of experiment so that each subject received all regimens at 24-hour intervals. Each regimen consisted of a single dose as follows: A, EDTA 380 mg. (disodium salt). B, Tetracycline HCl equivalent to 250 mg. of base. C, Tetracycline base 250 mg. D, Tetracycline HCl equivalent to 250 mg.

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of base plus 380 mg. EDTA. E, Tetracycline base 250 mg. plus 380 mg. EDTA. F, Ascorbic acid 500 mg. G, Tetracycline HCl equivalent to 250 mg. of base plus 500 mg. ascorbic acid. H, Tetracycline base 250 mg. plus 500 mg. ascorbic acid. Each drug was weighed out accurately and placed in capsules by one of the authors (G. M. E.).*

The method used to determine statistical significance of the differences between average values in small samples was that described by A. Bradford Hill.[6]

RESULTS

Serum antibacterial activity expressed as tetracycline HCl (micrograms per milliliter) for the various drug regimens is presented in Table I. Three values for Patient 6 are omitted because a small amount of antibacterial activity was detected in his serum collected prior to medication on the days of the indicated regimens.

TABLE I. SERUM ANTIBACTERIAL ACTIVITY EXPRESSED AS TETRACYCLINE HCl (μG PER ML.) THREE HOURS AFTER ORAL INGESTION OF MEDICATION

DRUG REGIMEN	SUBJECT NUMBER						AVERAGE
	1	2	3	4	5	6	
A. EDTA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. Tetracycline HCl	1.00	1.44	1.00	1.20	1.08	0.92	1.11
C. Tetracycline base	0.48	2.08	1.76	0.52	0.84	1.44	1.19
D. Tetracycline HCl + EDTA	0.33	0.40	0.52	0.32	0.60	--	0.43
E. Tetracycline base + EDTA	1.72	1.56	0.88	2.12	1.44	1.08	1.47
F. Ascorbic acid	0.00	0.00	0.00	0.00	0.00	--	0.00
G. Tetracycline HCl + ascorbic acid	0.56	1.28	2.04	0.56	0.76	2.44	1.27
H. Tetracycline base + ascorbic acid	0.88	0.60	0.28	0.72	0.60	--	0.62

TABLE II. STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN REGIMENS

REGIMENS COMPARED	STATISTICAL SIGNIFICANCE OF DIFFERENCES
B vs. C	Not significant
B vs. D	Significant
C vs. E	Not significant
D vs. E	Significant
B vs. G	Not significant
C vs. H	Not significant
G vs. H	Not significant

Oral administration of EDTA and ascorbic acid failed to confer detectable antibacterial activity upon the serum specimens. The average serum concentrations resulting from administration of tetracycline hydrochloride and tetracycline base were almost identical. When EDTA was given in conjunction with tetracyclines, there occurred a significant diminution of the serum tetracycline level in the case of the hydrochloride, whereas no significant alteration was observed in the case of the base. In contrast, when ascorbic acid was administered with either tetracycline base or hydrochloride, no significant diminution of the serum tetracycline level could be demonstrated.

*The tetracyclines used in this study were supplied by Dr. J. T. Groel, The Squibb Institute for Medical Research, New Brunswick, N. J.; ascorbic acid was supplied by Dr. N. H. Schimmel, Medical Division, Merck Sharp & Dohme, West Point, Pa.; and the EDTA was furnished by Dr. M. Seven, Department of Medicine, Hahnemann Medical College, Philadelphia, Pa.

Table II lists the statistical significance of the differences in average serum tetracycline concentrations resulting from the various drug regimens. It is noteworthy that the lowering effect on serum tetracycline concentrations of EDTA administered simultaneously with tetracycline hydrochloride was consistent, occurring in 5 out of 5 patients.

DISCUSSION

Although this investigation was limited to a small pilot study with determinations of serum antibiotic concentrations limited to only two points in time in relation to a single dose of medication, the results clearly indicate that EDTA tends to produce lower serum tetracycline concentrations than are obtained in the same patients when this complexing agent is not used. In addition, EDTA produces this effect only in combination with tetracycline hydrochloride and not with the base. To some extent these effects mimic the effect of dicalcium phosphate, although this compound does not discriminate between tetracycline hydrochloride or base, at least not in rats.[3]

It has been shown that dicalcium phosphate depresses absorption of tetracycline, as a consequence of which serum concentrations of the antibiotic are reduced.[2] It has also been reported that citric acid[2, 3] and sodium metaphosphate[2, 3, 7, 8] elevate serum tetracycline concentrations and it has been suggested that the latter compounds produce this effect by virtue of their ability to form complexes with calcium and thus counteract the depressant effect of calcium.[3] It might thus be assumed that EDTA, by virtue of its chelating activity for calcium, would be expected to act like citrate or metaphosphate and enhance serum tetracycline concentrations. This was found not to be the case under the conditions of the experiment herein described. As a matter of fact, a reverse effect was noted, as previously mentioned. Although we are unable at this time to offer an explanation for this wholly unexpected result, we are impressed by the need for further studies to elucidate the relationships between the tetracyclines, EDTA, and various metallic ions with respect to the absorption, transport, and excretion of tetracycline. It would be illogical to suppose that the absorption of tetracycline is uninfluenced by EDTA, since it has been established that EDTA is not absorbed from the gastrointestinal tract.

Tetracycline per se is a chelating agent and binds many metals, including aluminum and magnesium. It has been stated that the presence of these metals interferes with the absorption of tetracycline because the chelates thus formed are poorly absorbed.[1] It is possible that the effect of EDTA on serum tetracycline concentrations may be the result of competition between the two chelating agents for one or more metallic ions required for the transport of the antibiotic through the gastrointestinal mucosa into the blood stream.

Elevation of serum tetracycline concentration by ascorbic acid as reported by Freinberg and Lite[4] was not confirmed under the conditions of our study.

We would like to emphasize the fact that while the effects reported herein are interesting and challenging from the pharmacologic viewpoint, their significance in clinical therapy remains to be determined.

SUMMARY

Disodium EDTA causes a significant and consistent diminution in serum tetracycline concentrations 3 hours after oral administration of both drugs; however, this effect is seen only with the hydrochloride of tetracycline and not with the base. In contrast, it could not be shown that ascorbic acid significantly influenced serum tetracycline concentrations under the conditions in which it was employed.

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EFFECT OF A HYPOTENSIVE STEROID ON THE ACTION OF DESOXYCORTICOSTERONE IN THE RAT

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We recently described a new synthetic steroid, 17α-propyl-4,5β-dihydro-19-nortestosterone (SC-6584), that depressed the blood pressure of rats with the metacorticoid, metarenal, and adrenal-regeneration forms of hypertension.[1-4] Although metacorticoid hypertension is induced by temporary overdosage with desoxycorticosterone acetate (DCA) and sodium, the disease is sustained after treatment is interrupted.[5] Therefore, it was of interest to examine the effects of SC-6584 on blood pressure, electrolyte and water metabolism, and lesions in rats undergoing active treatment with DCA and sodium.

MATERIALS AND METHODS

Twenty-six male Sprague-Dawley rats, averaging 66 grams in weight, were placed in individual metabolism cages and assigned randomly to 4 groups. The rats were offered Rockland Rat Checkers and approximately normal (1 per cent) saline ad libitum. Urine was collected under toluene and measured at least every other day for volume and sodium and potassium concentrations. Saline intake, corrected for spillage, was measured similarly and each new batch was analyzed for sodium concentration. Body weights and indirect blood pressures[6] were recorded at intervals under "blind" conditions.

After 2 days of acclimatization, control measurements were taken over 8 days. Treatment then began as follows: Group A (6 rats), subcutaneous implantation of a single 40 mg. wax pellet; Group B (7 rats), implantation of a pellet containing 20 mg. wax plus 20 mg. DCA; Group C (6 rats), daily subcutaneous injections of SC-6584, 10 mg. per kilogram per day for 8 days, then double this dose for 36 days; Group D (7 rats), treatments B and C combined. After the last day of treatment, the survivors were sacrificed, and the various organs were weighed and examined histologically.

Statistical procedures involved ranking methods[7] instead of the more common standard error and "t" test methods because of uncertainty of homogeneity of variances and normality of distributions.

RESULTS AND DISCUSSION

During the course of treatment, one rat died in Group A and two in Group B. Body weight was not adversely affected by any of the treatments. Although every blood pressure reading was clear-cut and decisive, the results were quite variable on any given day and from day to day, probably as a result of the necessity of using untrained rats; nevertheless, there was statistically significant separation between Groups B and D on the last 2 days of observation (Table I).

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The blood pressure of Group B did not rise to the level expected on the basis of previous experience (175 to 200 mm. Hg). Because of this, plus the variability, we could not attach any great significance to the blood pressure data as a whole.

TABLE I. EFFECT OF SC-6584 ON PRESSOR ACTION OF DCA

GROUP AND TREATMENT	BLOOD PRESSURE (mm. Hg)		
	BEFORE INJECTION*	AFTER 13 DAYS	AFTER 29 DAYS
A. Wax pellet	141	134	160
B. DCA pellet	139	170	169
C. SC-6584	139	145	148
D. DCA + SC-6584	149	138†	150‡

*Average of 2 days.
†P = 0.01.
‡P = 0.035 that Groups D = B, by rank-sum test.

The temporal trends of fluid intake and output, sodium intake and output, potassium excretion, and the urinary sodium-potassium ratio were all very similar and, because of space limitations, they are exemplified in the graph for

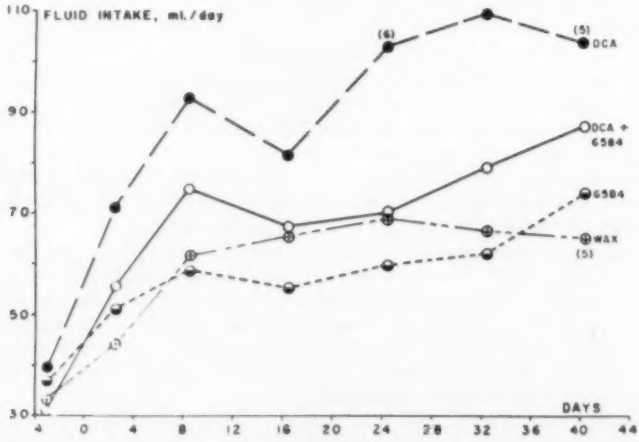

Fig. 1.—Effect of chronic treatment with SC-6584 on fluid intake of rats with DCA or wax pellet implants. Dosage (see text) was begun on day zero. Each group contains 7 rats unless otherwise noted in parentheses. Data are averaged over 4-day intervals.

fluid intake (Fig. 1). It is apparent that SC-6584 treatment inhibited the increases due to DCA; it also tended to depress the normal increases in those rats not treated with DCA. In all of the metameters there was a significant tendency for SC-6584 to act in the direction opposite to DCA in rats both treated and not treated with DCA. It is difficult to draw any causal inferences from the interrelationships among the various metameters because of the correlations that were uncovered. For example, the following correlations were statistically significant among all groups: sodium intake against sodium excretion and body weight; fluid intake against urine volume and body weight; sodium balance

against water balance, and both against potassium excretion; and sodium excretion against potassium excretion. It cannot be stated definitely whether the water and electrolyte effects of SC-6584 can serve to explain its hypotensive action in metacorticoid rats.[3]

The relative organ weights (Table II) revealed significant cardiorenal hypertrophy in the DCA-treated rats of Group D, but not in those of Group B. Such an effect was evident in the absolute weights for the latter, however. Treatment with SC-6584 had no apparent influence on organ weights. Histologically, both groups treated with DCA exhibited the well-known vascular and renal lesions, and SC-6584 had no effect on their incidence or severity; moreover, no toxic symptoms attributable to SC-6584 appeared in Group C. The inability of SC-6584 to prevent or reverse hypertensive lesions in the present case is consistent with similar findings in metarenal rats.[1]

TABLE II. RELATIVE ORGAN WEIGHTS (PER CENT)

GROUP	BODY WEIGHT (GM.)	ADRENALS	HEART	KIDNEYS	TESTES	THYMUS
A	286	0.029	0.41	0.93	1.07	0.21
B	304	0.025	0.40	1.00	1.06	0.19
C	344	0.024	0.34	0.80	1.03	0.19
D	321	0.028	0.48*	0.93*	0.95	0.17

*P = 0.02 that Groups D = B, by rank-sum test.

CONCLUSIONS

Chronic administration of the hypotensive steroid 17α-propyl-4,5β-dihydro-19-nortestosterone (SC-6584) in rats actively treated with DCA-saline tended to interfere with the pressor action of DCA, although these data were not impressive. The vascular and renal lesions due to DCA were not inhibited by the steroid. SC-6584 had a definite effect on water and electrolyte metabolism that was opposite to that of DCA in both DCA-treated animals and those not treated with DCA.

I am indebted to Dr. T. W. Harris for the histologic analyses and to Elizabeth Bosacki for her technical assistance.

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EVALUATION OF THE RADIOACTIVE (I^{131}-TAGGED) ROSE BENGAL LIVER FUNCTION TEST IN NONJAUNDICED PATIENTS

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THE rose bengal (tetrachlortetraiodofluorescein) test has been employed to a limited extent for clinical evaluation of hepatic function since its introduction by Delprat,[1] in 1923. The usefulness and limitations of this dye test are generally analogous to those of the test using sulfobromophthalein sodium (Bromsulphalein). After intravenous injection, the dye is presumed to be loosely bound to the plasma protein. Clearance of the dye from the blood takes place exponentially independent of its plasma concentration.[2] It is taken up in the liver apparently exclusively by the parenchymal cells,[3] and extrahepatic removal is not evident.[2] It is then excreted via the biliary tract into the intestine and eliminated in the feces. Enterohepatic circulation is absent.[4]

With the availability of rose bengal labeled with radioiodine (I^{131}), the possibility of dynamic measurement of hepatic uptake and excretion of the dye by means of external counting techniques appeared to offer a promising avenue of clinical estimation of hepatic function. Taplin and associates[4] inaugurated the liver function test employing radioactive (I^{131}-tagged) rose bengal in 1955. They found that animals with experimentally produced hepatocellular damage and with biliary obstruction showed definite and distinctive changes from normal in the pattern of uptake and excretion of the labeled dye as measured by an external counter placed over the region of the liver. They enthusiastically reported the results of applying this technique to the clinical evaluation of hepatic and biliary tract disease. It was their opinion that the test provided valuable diagnostic information in both jaundiced and nonjaundiced patients, that it was the only dye test which could be used safely in the presence of biliary tract obstruction, and that it was many times more sensitive than tests using nonradioactive dyes. In a later publication, they stated: "The Rose Bengal I^{131} Hepatogram is presently the most sensitive specific test for polygonal cell function of the liver . . . ."[5]

The initial enthusiasm generated by the reports of Taplin's group was nurtured by the subsequent favorable studies of Marshall and Kozoll,[6] and of Blahd and Nordyke.[7] However, Brown and Glasser,[8] as well as Rosenberg and co-workers,[9] reported less favorable data. The latter group found that results of the rose bengal-I^{131} test were normal in 2 of 12 patients who had active hepatic disease and greatly abnormal function; of 8 patients with only minimal signs of hepatic disease, 7 had normal curves and the eighth had a borderline curve.

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The earlier methods of interpretation of the uptake and excretion curves of rose bengal-I^{131} involved qualitative visual comparison of each curve with a group of normal curves. Later attempts at quantitative evaluation involved comparison of peak levels of radioactivity or comparison of the height of the curves at different intervals after injection. The results of such analyses were subject to a great many uncontrollable variables, including the size of the patient, the mass of the liver, the thickness of the body wall interposed between the liver and the counter, and the exact position of the hepatic mass in relation to the counter. It is possible that such variations could render the test questionable, if not totally unreliable.

In an attempt to correct these potential sources of error, Lowenstein[10] devised an ingenious mathematical method of purely quantitative analysis of the uptake-excretion curve. He reasoned that the radioactivity recorded over the liver at any point in time was equal to the difference between the amount of activity taken up and the amount excreted. Therefore, each curve could be resolved into two component curves, one representing uptake and the other excretion. Since both the uptake and the excretion of the dye change logarithmically with time, both component curves would be straight lines when plotted on semilogarithmic paper and could be expressed numerically.

The mechanics of Lowenstein's method are shown in Fig. 1. After the background activity is subtracted, the curve is transposed to semilogarithmic paper. The descending limb of the curve is taken to represent biliary excretion after all the dye has been taken up by the liver. Therefore, a line drawn through the descending limb and extended to the ordinate would represent the entire excretion component of the curve (E). Points on the uptake component of the curve (U) then may be obtained by subtracting the values of the rising portion of the curve from the corresponding values of the excretion component (E). Each of these lines is characterized numerically. Lowenstein chose to use the figures for half-uptake time (T_u), which is the period during which the liver takes up one half of the total circulating dye from the blood, and for half-excretion time (T_e), which is the period during which one half the total dye is excreted from the liver. The difference between line E and line U at the ordinate would be proportional to the fractional hepatic blood volume.

After using this method of analysis on 42 curves of Taplin and associates and on 28 of his own, Lowenstein concluded that hepatocellular damage is manifested by delayed uptake and slightly delayed excretion, whereas obstructive processes are characterized by greatly delayed excretion. He considered that the values obtained in various hepatic diseases were characteristic of the disease and indicated whether cellular damage or obstruction predominated.

The primary purpose of the present study was to determine the relative value of the liver function test using rose bengal-I^{131} in the diagnosis of hepatocellular damage in nonjaundiced patients by means of the technique devised by Taplin and co-workers and the method of analysis recommended by Lowenstein. Particular attention was directed to comparison of the sensitivity and clinical usefulness of this test with those of the standard Bromsulphalein test.

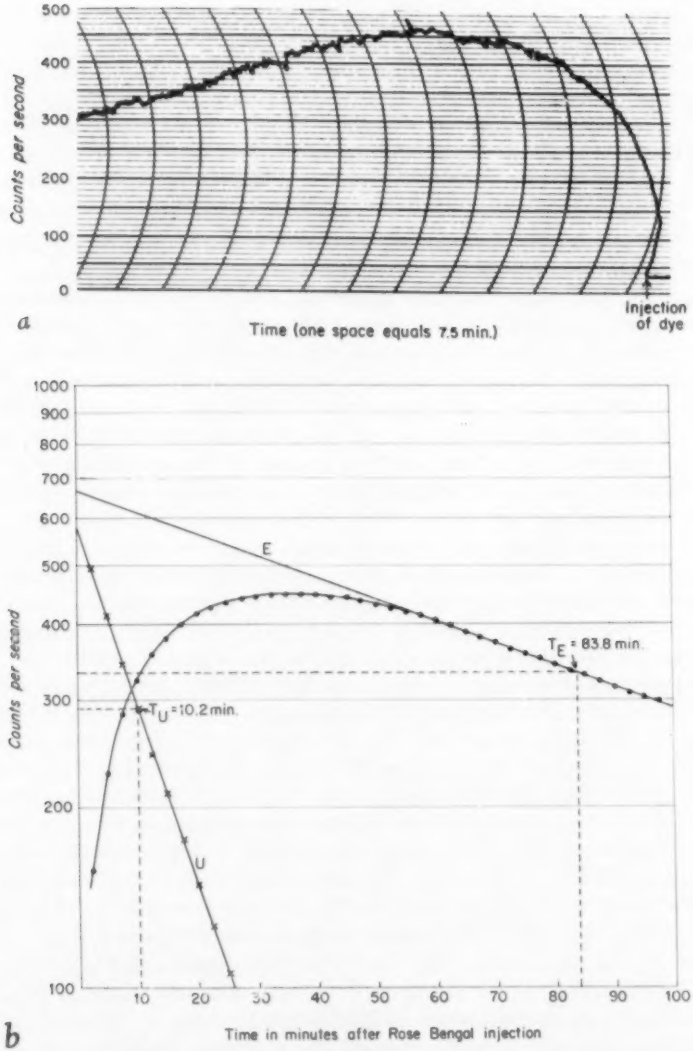

Fig. 1.—Calculation of half-uptake time (T_u) and half-excretion time (T_e) of rose bengal-I^{131}. *a*, Recorded uptake-excretion curve in a normal person (record is made from right to left). *b*, Curve transposed to semilogarithmic paper and resolved into uptake (U) and excretion (E) components, with determination of T_u and T_e. See text for details.

MATERIALS AND METHODS

Commercially prepared I^{131}-labeled rose bengal* was diluted with an isotonic solution of sodium chloride both with and without the addition of nonradioactive dye. Each dose

*Use of the I^{131} was authorized by the Atomic Energy Commission.

contained a total radioactivity of 15 μc. The dose of dye varied between 0.2 and 10 mg. As has been previously demonstrated by Taplin and associates,[4] varying the dose over this relatively wide range does not affect the test significantly.

With the patient reclining supine on an examining table, a collimated, 5 cm. sodium iodide scintillation counter was positioned over the zone of hepatic dullness and angled away from the region of the gall bladder. The aperture viewed was an 8 cm. circle at the cutaneous surface. After the background activity was determined, the test dose was administered intravenously. The patient then was required to lie in position under the counter for 1 to 2 hours while a continuous recording of the uptake and excretion of the labeled dye was made on an Esterline-Angus recorder.

After correction for background activity, the tracings were plotted on semilogarithmic paper. Half-uptake times (T_u) and half-excretion times (T_e) were calculated according to the method of Lowenstein.

RESULTS

The test using rose bengal-I^{131} was done on 30 patients. Eleven patients were classified as controls; these included patients in good general health and patients with gastrointestinal disease having no apparent effect on hepatic function, such as uncomplicated duodenal ulcer and diaphragmatic hernia. The remaining 19 patients were classified as having hepatic disease without jaundice; these included 8 patients with Laennec's cirrhosis, 3 with postnecrotic cirrhosis, 2 with secondary biliary cirrhosis, 2 with metastatic malignant tumors, 2 with hemochromatosis, 1 with brucellosis, and 1 with constrictive pericarditis. All these patients had both clinical and laboratory evidence of hepatic disease. All except the 2 with hemochromatosis and the 1 with brucellosis had significant degrees of retention of Bromsulphalein.

TABLE I. RATE OF UPTAKE (T_u) OF RADIOACTIVE ROSE BENGAL IN 19 PATIENTS WITH SPECIFIC HEPATIC DISEASES

HEPATIC DISEASE	T_u (MINUTES)	
Laennec's cirrhosis	6.0	Normal Range
Laennec's cirrhosis	6.5	
Hemochromatosis*	6.5	
Posthepatitic cirrhosis*	6.8	
Laennec's cirrhosis	7.5	
Secondary biliary cirrhosis*	8.5	
Metastatic carcinoma (prostate)	9.0	
Metastatic melanoma	10.5	
Posthepatitic cirrhosis	12.6	
Laennec's cirrhosis*	13.0	
Posthepatitic cirrhosis*	13.5	
Laennec's cirrhosis	13.5	
Brucellosis*	13.5	
Cardiac cirrhosis* (constrictive pericarditis)	14.5	
Hemochromatosis*	15.5	
Laennec's cirrhosis	17.5	
Laennec's cirrhosis	21.7	
Laennec's cirrhosis	22.0	
Secondary biliary cirrhosis	34.0	

*Confirmed at surgical exploration or percutaneous hepatic biopsy.

In Fig. 2, the half-uptake times (T_u) in the control group are compared to those of the patients with hepatic disease. The mean T_u for the patients

with hepatic disease (13.3 minutes) is distinctly longer that that of the controls (9.3 minutes). However, 8 of the 19 patients (42 per cent) with hepatic disease had half-uptake times within the range of the controls (6 to 12 minutes).

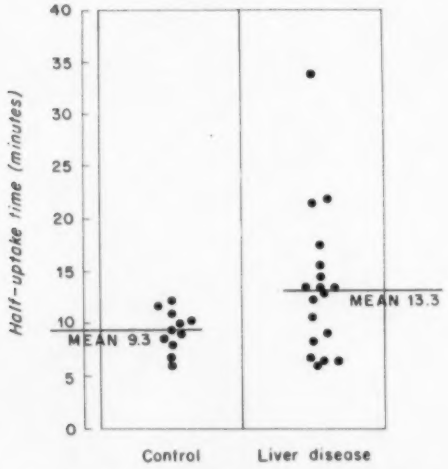

Fig. 2.—Half-uptake time (T_u) of rose bengal-I^{131} in control patients and in patients with hepatic disease.

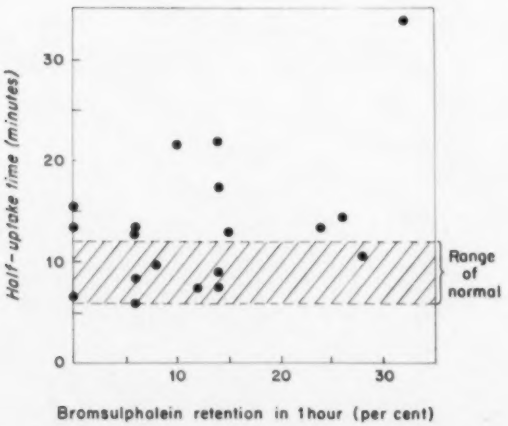

Fig. 3.—Comparison of half-uptake time (T_u) of rose bengal-I^{131} with degree of retention of bromsulphalein in 19 patients with hepatic disease.

The half-uptake times determined in these 19 patients with specific diseases of the liver are shown in Table I. No indication exists that the sensitivity of the test with labeled rose bengal is increased in any specific hepatic disease state or that the test would be helpful in differentiating specific types of hepatic disease in nonjaundiced patients.

In Fig. 3, the half-uptake times of the patients with hepatic disease are plotted against the degree of retention of Bromsulphalein. The mean half-uptake time of rose bengal-I^{131} tends to increase as the retention of Bromsulphalein increases; however, considerable overlap occurs between values in the normal group and values in the groups with minimal and even pronounced retention of Bromsulphalein.

There were only 2 patients with no retention of Bromsulphalein who had moderately prolonged half-uptake times for labeled rose bengal; one had brucellosis and the other had hemochromatosis. A second patient with hemochromatosis had values well within the normal range for both tests.

Values of the half-excretion time (T_e) did not aid in discrimination between the control group and the patients who had hepatic disease; the mean values were 110 and 122 minutes, respectively, with a large overlap in the individual values for each group.

Other methods of comparison of the uptake-excretion curves of the control patients and the patients with hepatic disease were grossly insensitive. Visual comparison of the two groups of curves, as well as comparison of peak-count values, was decidedly less sensitive than was comparison of the half-uptake times.

COMMENT

Since rose bengal is removed from the blood apparently exclusively by the hepatic parenchymal cells, this dye test may be considered as a specific measure of the function of the polygonal cells, and as such it has proved to be a useful tool in the experimental laboratory. Despite this specificity, however, the test with unlabeled dye has been found lacking in sensitivity. For this reason, it has been largely abandoned for clinical use in favor of the more discriminating Bromsulphalein test. This lack of sensitivity was well demonstrated by Monroe and Hopper,[11] who studied 24 patients with clinical evidence of hepatic disease using both unlabeled rose bengal and Bromsulphalein tests on each patient. Ten of these 24 patients (42 per cent) had positive results with Bromsulphalein and normal results with rose bengal; 2 other patients had greatly abnormal results with Bromsulphalein and only borderline retention of rose bengal. No patients in their group had positive results with rose bengal and a normal Bromsulphalein test, and only one had borderline results with rose bengal and a normal Bromsulphalein test. Similar experiences with the lack of sensitivity of the test using unlabeled rose bengal led to its abandonment as a clinical test of hepatic function at the Mayo Clinic.[12]

In the diagnosis of hepatic disease without jaundice, the most important information to be gained from the test with rose bengal-I^{131} appears to be the rate at which the liver clears the dye from the blood. This is precisely the same information that is obtained in a more direct manner from the use of nonradioactive dye in this test. There appears to be no reason why the addition of a radioactive tag should improve the sensitivity of the same basic procedure.

In addition to its lack of sensitivity, the test employing rose bengal-I^{131} presents the following practical disadvantages as a clinical test for hepatic function in nonjaundiced patients: (1) each test ties up costly equipment for 1

to 2 hours; (2) the patient is required to maintain the same position under the counter for 1 to 2 hours, which often causes discomfort during this prolonged period of enforced immobility; (3) interpretation of curves according to the method of Lowenstein (transposing the curve to semilogarithmic paper and calculating T_u and T_e) is a time-consuming procedure requiring the attention of a highly trained technician.

Thus, it appears that the radioactive rose bengal test of liver function in nonjaundiced patients is insensitive and impractical when compared with the Bromsulphalein test. As a research technique in experimental hepatic disease,[13] it may well be valuable. At the present time, the reports of clinical experience with this test in jaundiced patients are not sufficiently extensive to permit evaluation of its usefulness.

SUMMARY

The liver function test utilizing radioactive (I^{131}-tagged) rose bengal was used in the evaluation of hepatic function of 30 nonjaundiced patients. Uptake-excretion curves were obtained according to the technique introduced by Taplin and associates and were analyzed according to the method described by Lowenstein.

Whereas the average rate of hepatic uptake of the dye was slower in patients with hepatic disease than it was in control patients, a large overlap occurred between the individual values of the group with hepatic disease and those of the control group. Because of this lack of sensitivity and because of practical difficulties in its performance, the test employing radioactive rose bengal cannot be recommended for routine clinical use in evaluation of hepatic function in nonjaundiced patients.

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CRITICAL EVALUATION OF THE PRECIPITATION TEST FOR SYSTEMIC LUPUS ERYTHEMATOSUS

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RECENTLY, Jones and Thompson[1] have published a simple precipitation test for systemic lupus erythematosus. The test is carried out by adding a 12 per cent solution of *p*-toluenesulfonic acid in glacial acetic acid to serum. The authors claimed the test to be specific for systemic lupus erythematosus and stated that they had found very few false positives. These false positive reactions were found in cases of hepatitis, in disseminated coccidioidomycosis, and in two cases of fulminating multiple myeloma.

It is well known that an increase of the gamma globulin fraction in the serum is often found in systemic lupus erythematosus. This also is the case in the diseases that gave false positive reaction. To test the hypothesis that the precipitation test is a nonspecific reaction, the following experiments were carried out.

MATERIALS AND METHODS

One hundred and fifty-eight serum samples were examined. All had been sent to the Department of Clinical Biochemistry for various routine analyses from the different departments of the Karolinska Hospital.

In the experiment recorded in Table III a batch of pooled normal human serum was used. This serum was negative when tested with the precipitation test under investigation. Lyophilized human gamma globulin,* prepared from retroplacental blood, was dissolved in this serum and a series of dilutions were made, resulting in a number of serum samples with increasing gamma globulin contents.

Precipitation Test.—The test was done exactly according to the description by Jones and Thompson: 0.1 ml. of serum was placed in a test tube, and 2 ml. of a 12 per cent solution of *p*-toluenesulfonic acid in glacial acetic acid was added. The tube was shaken, allowed to stand for 20 minutes, and then reshaken. The result was estimated as follows. (1) If no clot or precipitate was seen after the addition of the test reagent or if it disappeared on shaking, the reaction was termed negative. (2) If a precipitate persisted 20 minutes after shaking and standing but disappeared on reshaking, the reaction was termed 1+. (3) If a precipitate persisted after standing 20 minutes and reshaking, the reaction was termed 2+. (4) If the precipitate was heavy, the reaction was termed 3+.

Paper Electrophoresis.—Paper electrophoresis was done on all serum samples. Barbital buffer of pH 8.6 and ionic strength 0.1 was used. The separation was performed for 18 hours with a current of 5 Ma. The strips were stained with bromphenol blue, cut, and the dye eluted with a sodium carbonate and methanol solution. The concentration of dye was determined by measuring the optical density in a Beckman B spectrophotometer at 595 mμ.

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*The gamma globulin was kindly provided by A. B. Kabi, Stockholm, Sweden.

Protein Determination.—Total protein was determined by the Van Slyke copper sulfate method.[2] If the protein content was found to be lower than 5.5 Gm. per 100 or higher than 8.0 Gm. per 100, a Kjeldahl determination was performed. In the experiment with added gamma globulin, the total protein was determined by the biuret method.[3]

RESULTS AND DISCUSSION

Table I shows the result of the precipitation test correlated with the relative content of gamma globulin of the serum examined. It is seen that a positive test is very unusual if the gamma globulin is below 20 relative per cent and that the number of positive tests increases with increasing gamma globulin content. The same results were obtained when the number of positive tests was correlated with the absolute instead of the relative amounts of gamma globulin. The results of the test could not be correlated with changes of any of the other globulin fractions in serum.

TABLE I. RESULT OF THE PRECIPITATION TEST CORRELATED WITH THE GAMMA GLOBULIN CONTENT IN SERUM

GAMMA GLOBULIN AS PER CENT OF TOTAL PROTEIN	POSITIVE TESTS			TOTAL POSITIVE TESTS		NEGATIVE TESTS		TOTAL
	+	++	+++	NUMBER	PER CENT	NUMBER	PER CENT	
< 19.9	1			1	1	72	99	73
20.0 – 24.9	7	3	2	12	26	34	74	46
25.0 – 29.9	1	4	1	6	33	12	67	18
30.0 – 34.9	1	6		7	54	6	46	13
35.0 – 39.9		4	1	5	83	1	17	6
40.0 >			1	1		1		2
	10	17	5	32		126		158

Thirty-two positive tests were found out of 158 performed, i.e., 20.3 per cent. The distribution of these positive tests with regard to the strength of the precipitation is shown in Table I. There seems to be a tendency toward a heavier precipitation with increasing gamma globulin content.

TABLE II. DIAGNOSES IN CASES WITH POSITIVE PRECIPITATION TEST

DIAGNOSIS	NUMBER OF CASES
Rheumatoid arthritis	7
Hepatic cirrhosis	5
Elevated E.S.R.	4
Diffuse joint troubles	3
Systemic lupus erythematosus	2
Ulcerative colitis	2
Multiple myeloma	1
Fever of unknown origin	1
Miscellaneous	7
Total	32

Table II shows the established diagnoses in the cases with positive tests. It is evident that the test gives positive results in rheumatoid arthritis and hepatic cirrhosis, as well as in ulcerative colitis and multiple myeloma. All these chronic diseases often have increased gamma globulin fractions in serum.

Four cases were under examination for an elevated erythrocyte sedimentation rate and 3 for diffuse joint troubles. All had elevated gamma globulin fractions, but no L.E. cells could be demonstrated. It should be noted that 4 out of 6 cases of proved systemic lupus erythematosus showed negative tests.

Table III shows the result of the experiment with gamma globulin added to pooled normal serum. It is seen that the originally negative serum turned positive when gamma globulin was added. Also the precipitation apparently was heavier with increasing amounts of gamma globulin added.

TABLE III. PAPER ELECTROPHORETIC PATTERNS AND RESULT OF PRECIPITATION TEST AFTER ADDITION OF GAMMA GLOBULIN

EXPERIMENT NO.	PAPER ELECTROPHORESIS					TOTAL PROTEIN	PRECIPITATION TEST
	ALBUMIN	α_1	α_2	β	γ		
1	60.3	4.7	11.8	6.4	16.8	6.37	−
2	58.9	3.8	11.5	5.6	20.2	7.04	+
3	51.4	4.7	9.8	5.6	28.5	7.78	++
4	51.8	4.3	8.6	5.0	30.3	8.33	++
5	47.5	3.8	8.1	4.8	35.8	9.15	+++
6	45.1	3.8	7.8	4.5	38.8	9.75	+++
7	41.8	3.0	9.6	4.8	40.8	10.07	+++

These results all seem to indicate that the precipitation reaction is not specific for systemic lupus erythematosus, but is a gamma globulin reaction. Thus it should be classified together with other nonspecific gamma globulin reactions, such as the Takata Ara reaction and the zinc sulfate reaction.

SUMMARY

Examination of 158 sera with the precipitation test of Jones and Thompson seemed to indicate that the test was not specific for systemic lupus erythematosus but dependent on the gamma globulin content of the serum.

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LABORATORY METHODS

A SPECIFIC METHOD FOR THE DETERMINATION OF SERUM IRON

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RECENT investigations in copper metabolism in this and other laboratories have strongly suggested an interrelationship with iron metabolism. However, further investigation was hindered because of the inadequacy of existing methods for the determination of iron.

In our screening of available methods for serum iron determination, it was found that the other metal ions present interfered. Copper ion was especially troublesome and since the concentration of copper and iron in serum are roughly equal, serious errors were thus introduced. We, therefore, sought a reagent which would be specific for iron. It was found that 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) satisfied our requirements since it is quite specific for ferrous ion at a pH of 4 and forms stable color complex which is completely extracted with isoamyl alcohol.[1] This reaction follows Beer's law, giving a linear curve. In this laboratory, a standard curve gave a density reading of 0.065 for each gamma of iron in a 5 ml. isoamyl alcohol solution.

Utilizing this reaction, a simple, rapid, and accurate method was developed which reacts only with iron and is not interfered with by other transition group metals normally present in biologic fluids.

PROPOSED METHODS

Apparatus.—A Beckman DU spectrophotometer operating at a wave length of 534 mμ and utilizing 1 cm. cells was employed for all spectrophotometric determinations.

Reagents.—1. Iron-free water obtained by redistillation in an all-glass apparatus.
2. Hydrochloric acid, 2N.
3. Trichloroacetic acid, 20 per cent (weight by volume).
4. Bathophenanthroline, 0.001 M solution (0.0332 Gm. in 50 ml. of ethyl alcohol and diluting with 50 ml. of iron-free water).
5. Hydroxylammonium chloride, 10 per cent (weight by volume).
6. Sodium acetate, 10 per cent (weight by volume).
7. Isoamyl alcohol (reagent grade).

All solutions were prepared with iron-free reagents and solvents. All glassware was treated with the usual precautions used in trace metal determinations.

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Preparation of blood serum: Serum iron was liberated by the acid incubation method of Gubler and associates[2] which is very satisfactory.

Serum: To 1 ml. of serum in a 15 mm. test tube, add 1 ml. of 2N hydrochloric acid, stir well, and let stand for 10 minutes. Then add 1 ml. of 20 per cent trichloroacetic acid solution, stir well, and let stand for another 10 minutes. Cover tubes with polyethylene caps and centrifuge at 3,000 r.p.m. for 30 minutes to ensure maximum yield of supernatant. Add 3 ml. of 10 per cent sodium acetate solution and 2 ml. of 10 per cent hydroxylammonium chloride and stir gently. Then add 1 ml. of the bathopenanthroline solution, stir well, and let stand for 5 minutes with occasional stirring. Add 5 ml. of isoamyl alcohol, cover with polyethylene caps, and shake well for 2 minutes. Centrifuge for 5 minutes to accelerate the separation of the two phases. The perfectly clear isoamyl alcohol layer is read against a reagent blank at 534 mμ and compared with the standard curve.

Other biologic materials were prepared by perchloric acid digestion and analyzed in a similar fashion.

TABLE I. MULTIPLE DETERMINATIONS OF SERUM IRON ON THE SAME SPECIMEN

IRON CONTENT (MICROGRAMS PER 100 ML.)	
SERUM A	SERUM B
(1) 123	(1) 225
(2) 125	(2) 226
(3) 118	(3) 228
(4) 115	(4) 220
(5) 122	(5) 226

TABLE II. RECOVERY OF ADDED IRON TO SERUM SPECIMEN

SERUM	IRON CONTENT (MCG./100 ML.)	IRON ADDED (MCG./100 ML.)	IRON FOUND (MCG./100 ML.)
A	132	100	238
	134	200	330
	138	300	428
B	225	100	332
	228	200	420
	233	300	523

TABLE III. COMPARISON OF WET ASH METHOD WITH DESCRIBED METHOD

SERUM	WET ASH (MCG./100 ML.)	NEW METHOD (MCG./100 ML.)
A	165	172
B	225	216
C	275	282
D	150	148
E	195	188

DISCUSSION

The data in Table I show that the reproducibility of this method is well within analytic standards. In Table II, the data show that this method consistently demonstrates good recovery of added iron.

Inasmuch as we were not content with any of the existing methods for the determination of serum iron, we decided to compare our findings with those of the wet ash method of analysis. The results are presented in Table III. These data illustrate that our method compared favorably with the wet ash technique.

SUMMARY

A rapid, simple, specific, and highly accurate method for the determination of iron in biologic media has been presented. It has the advantage of not reacting with other metal ions normally present in biologic substances and which interfere with techniques currently in use. Only 1 ml. of serum is necessary for the test. Protein-bound iron is liberated by Gubler and associates' acid incubation method, the supernatant fluid is reacted with bathophenanthroline, and the color complex is extracted with isoamyl alcohol. The color density is read in a Beckman DU spectrophotometer and compared with a standard curve.

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Erratum

In the article by Ernest Beutler, M.D., and Richard K. Blaisdell, M.D., entitled "Iron Enzymes in Iron Deficiency. III. Catalase in Rat Red Cells and Liver With Some Further Observations on Cytochrome C," which appeared in the November issue of the JOURNAL, pages 694-699, Reference 2 on page 699 should read as follows: 2. Beutler, E.: Iron Enzymes in Iron Deficiency. I. Cytochrome C, Am. J. M. Sc. **234**: 517-527, 1957.

THE COUNTING OF BARIUM CARBONATE IN A LIQUID SCINTILLATION SPECTROMETER

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THE measurement of the specific activity of C^{14} is of importance in a number of clinical and biochemical investigations. Small amounts of compounds containing C^{14} of high specific activity may be either converted to barium carbonate, plated, and counted in a flow gas counter or plated and counted directly. Large samples of low specific activity pose a more difficult problem. The use of the ionization chamber for the assay of such samples provides sensitivity and reproducibility, but the technique is laborious and time consuming. The liquid scintillation counter is extremely useful for the measurement of weak beta emitters of low specific activity, but until recently only compounds which are soluble in a limited variety of hydrocarbon solvents were counted with this instrument. The introduction of scintillating gels by Funt[1] and the details provided by White and Helf[2] have appreciably broadened the applicability of liquid scintillation counting.

The purpose of this paper is to describe a simplified method of counting large samples of $BaC^{14}O_3$ of low specific activity in a thixotropic scintillator gel. This technique has largely supplanted the ionization chamber for the counting of $C^{14}O_2$ in this laboratory.

METHODS

$C^{14}O_2$ is prepared from an organic material by the Van Slyke-Folch procedure.[3, 4] The $C^{14}O_2$ is trapped in sodium hydroxide, and barium carbonate is precipitated by the addition of barium chloride. The crystals are dried at 200° C. and ground to a fine powder with a mortar and pestle. Sieving of the powder as recommended by White and Helf has been found to be unnecessary. A carefully weighed amount of $BaCO_3$ is added to a 5 dram counting vial* and 20 ml. of the scintillator gel† is syringed into the vial. The vial is shaken vigorously until the particles are evenly dispersed throughout the gel. It is then placed in the deep freeze until it is counted in a liquid scintillation spectrometer.[5] The count rate per milligram of $BaCO_3$ is corrected for self-absorption (see below).

RESULTS

The Reproducibility of Gel Counting of $BaCO_3$.—A single sample of standard $BaC^{14}O_3$ was thoroughly ground and equal aliquots added to each of 5

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*No. 3305 Snap-Cap, T. C. Wheaton Company, Millville, N. J.

†The gel is composed of toluene, Thixcin (25 Gm./L.), 2,5-diphenyloxazole (3.0 Gm./L.), and 1,4-di(2,5-phenyloxazolyl)benzene (0.6 Gm./L.). These ingredients are blended in an ordinary Waring blendor for about 5 minutes until a viscous gel is formed. The 1,4-di(2,5-phenyloxazolyl)benzene is optional depending on the spectral sensitivity of the particular photomultiplier tubes.

counting vials. The count rates are shown in Table I and indicate that reproducibility of a given sample is, for practical purposes, limited by counting statistics. A single standard sample has maintained its count rate for several months despite periodic active shaking.

Since $BaCO_3$ is a powdery substance it tends to conglomerate within the gel. This suggested the possibility of poor reproducibility due to unequal grinding with resultant differences in self-absorption. To evaluate this variable, 5 suspensions of a single $BaC^{14}O_3$ standard were individually filtered, dried, ground, and counted. The results are shown in Table II and again indicate that reproducibility of sample preparation is either limited by or only slightly larger than the inherent statistical error.

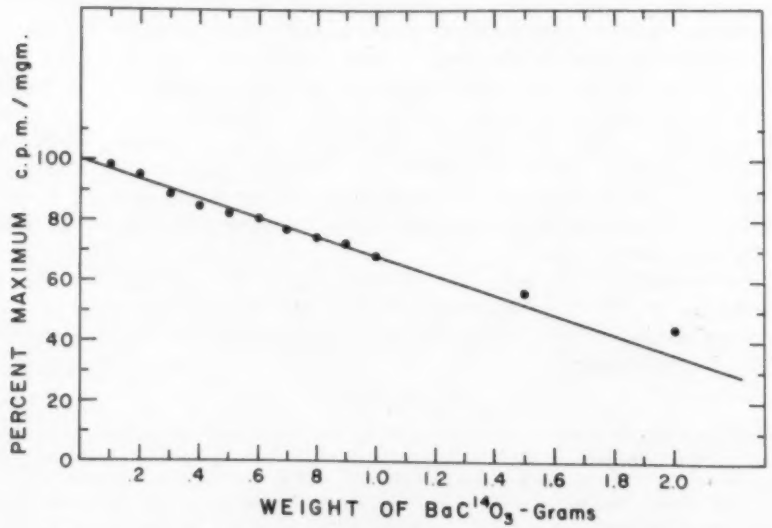

Fig. 1.—The effect of increasing weight of $BaC^{14}O_3$ on the observed specific activity.

The Effect of the Amount of $BaCO_3$ on the Count Rate.—White and Helf[2] showed that the counting efficiency for certain C^{14}-labeled compounds decreased with increasing quantities of the substances. To evaluate this aspect of the above method, increasing quantities of $BaCO_3$ were counted. The results of this study are plotted in Fig. 1. The straight line between 0.1 and 1.0 Gm. is extrapolated to zero and provides the necessary data for correction of samples for self-absorption if the sample weight is restricted to 1.0 Gm. or less.

Counting Efficiency.—Standard 200 mg. samples of $BaC^{14}O_3$ were counted by the above technique and smaller amounts were counted in a proportional flow gas counter of known efficiency. The efficiency of the scintillator system proved to be 56 per cent for 200 mg. of $BaCO_3$.

TABLE I. THE SPECIFIC ACTIVITIES OF 5 REPLICATES OF A GROUND SAMPLE OF $BaC^{14}O_3$

SAMPLE NUMBER	C.P.M. PER MG. $BaCO_3$	PER CENT DEVIATION FROM THE MEAN	PER CENT STATISTICAL COUNTING ERROR (2 STANDARD DEVIATIONS)
1	52.3	0.2	1.4
2	52.7	0.6	1.4
3	52.5	0.2	1.4
4	51.9	0.6	1.4
5	52.6	0.4	1.4

TABLE II. THE SPECIFIC ACTIVITIES OF 5 SAMPLES OF A SINGLE STANDARD $BaC^{14}O_3$ WHICH HAVE BEEN SEPARATELY FILTERED, DRIED, AND GROUND

SAMPLE NUMBER	C.P.M. PER MG. $BaCO_3$	PER CENT DEVIATION FROM THE MEAN	PER CENT STATISTICAL COUNTING ERROR (2 STANDARD DEVIATIONS)
1	29.4	1.0	0.8
2	29.7	2.0	0.8
3	29.0	0.3	0.8
4	28.5	2.0	0.8
5	29.1	0	0.8

DISCUSSION

The described technique is simple, efficient for C^{14}, and rapidly performed. Six hundred samples have been counted over a period of a few months in this laboratory. The counting efficiency is considerably higher than that of a recently described technique for counting equal amounts of $C^{14}O_2$.[6]

Despite the apparent gross opacity of the sample gels and variability of the size and shape of the conglomerates of $BaCO_3$, reproducibility of both sample preparation and sample counting falls largely within the error of counting statistics.

SUMMARY

1. A method for counting relatively large amounts of low specific activity $BaCO_3$ has been described.

2. The technique is simple, adaptable, and reproducible with an efficiency of 56 per cent for 200 mg. of $BaCO_3$.

3. This technique obviates the need for low efficiency "infinite thickness" counting and replaces ionization chamber counting for most samples.

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ABSTRACTS

64. EVALUATION OF SPIROMETERS IN PULMONARY FUNCTION TESTING, WITH OBSERVATIONS ON THE EFFECT OF THE BREATHING RATE ON THE MAXIMUM BREATHING CAPACITY

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Stead and Wells have recently demonstrated with a mechanical pump system that the responses of conventional counter-weighted metal bell spirometers were inaccurate when the pump frequency exceeded 80 per minute. They have designed a new direct writing spirometer with a reduced dead space and a lightweight plastic bell. In order to assess the significance of the bell type on ventilatory testing, we have made comparisons of the metal and plastic bell spirometers in single breath and maximum breathing capacity (MBC) measurements in patients and normal subjects.

No differences could be demonstrated between the two types of spirometers for the 0.5, 1.0, and 3.0 sec. expiratory capacities nor for the total vital capacity. The mean maximal expiratory flow rate (MEFR) was 1.9 liters per second greater ($p<0.01$) and the mean maximal mid-expiratory flow rate (MMF) was 0.17 liters per second smaller ($p<0.02$) with the metal bell spirometer, probably because of the greater mass of the metal bell system.

In 13 comparisons of the two spirometer types in patients with pulmonary disease, no differences between the two spirometers could be demonstrated in either single breath or MBC measurements. In 10 normal subjects the MBC was measured on both spirometers with the aid of a metronome at graded breathing rates from 10 to 180 breaths per minute. No effect of bell type could be seen for maximum breathing capacities performed at breathing rates below 120 per minute. At higher breathing rates the MBC values were 10 to 15 per cent higher for the metal bell ($p<0.01$).

Irrespective of spirometer type, the true MBC in normal subjects could not be attained with certainty at breathing rates below 90 per minute. At higher breathing rates the MBC remained relatively constant with the plastic bell spirometer.

It may be concluded that the optimum breathing rate for performance of the MBC in normal subjects is 90 to 110 breaths per minute, and that at this breathing rate the 13½ L. conventional metal bell spirometer will accurately record the MBC.

65. THE USE OF RADIOACTIVE TRIOLEIN AND OLEIC ACID IN THE DIFFERENTIATION OF DISTURBANCES OF FAT DIGESTION AND FAT ABSORPTION

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Since the recent availability of both glycerin trioleate and oleic acid tagged with iodine^{131}, a gamma-emitting radioisotope, hopes have been raised for the development of a simple, quick, inexpensive, and accurate method of measuring intestinal digestion and absorption of fat.

Our method consisted of administering, in the morning after an 8-hour fast, a gelatin capsule containing a 50 μc dose of tagged triolein and then, on the following day, tagged oleic acid. Each capsule was given with a test meal made up of 0.5 ml. peanut oil per kilogram body weight, one ounce chocolate syrup for palatability, made up to 240 ml. with cold milk and emulsified in a blendor. Blood samples were withdrawn at 1½, 3, 4, 5, and 6 hours. The radioactivity per ml. in each sample was measured in a well-type scintillation counter. The subject's blood volume was calculated and the blood levels of radioactivity were expressed as per cent of the ingested dose.

Deviations from normal were easily discerned. Digestive disorders could be separated from primary absorptive disorders because fats must be hydrolyzed by digestive enzymes to glycerin and fatty acids before absorption may occur, whereas fatty acids are absorbed without the need for digestive enzymes. Thus, when digestive function is inadequate, fatty acids are absorbed in normal amounts but fats are not. If primary absorption defects are present, neither fat nor fatty acid will be absorbed in normal amounts.

Utilizing this technique in 36 patients, normal curves of digestion and absorption were established in 13 control patients and 10 peptic ulcer patients prior to gastric surgery; this latter group is to be followed with postoperative studies. Of 8 patients with megaloblastic anemia, 3 showed defects in digestion and 3 showed defects in absorption. Of 3 cases of steatorrhoea, two showed digestive defects. A case of pancreatic resection had normal absorption, and a case of partial pancreatic cutaneous fistula had normal digestion and absorption.

This method promises to be useful in the study of nutritional problems resulting from surgery and in the differentiation of the steatorrhoeas.

66. EXCHANGEABLE C^{14}-CHOLESTEROL POOL SIZE AS AN INDEX OF CHOLESTEROL METABOLISM: EFFECT OF LOW FAT AND HIGHLY UNSATURATED FAT DIETS

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Five patients with known atherosclerotic heart disease were transferred to a metabolic ward and given a diet of normal fat content (35 per cent of total calories). Following 3 weeks of this diet, 2 patients ate an 11 per cent fat diet for an additional 4 weeks. One of these 2 patients, and an additional 3 patients who had been eating a normal fat diet for 2 weeks, were then given a diet containing large amounts of linoleic acid for 3 weeks. The last 3 patients ate this as a liquid formula diet containing only safflower oil as a source of fat.

Ten days prior to the termination of each diet period, patients were injected intravenously with 10 μc of cholesterol-4-C^{14}. Blood samples, taken every 2 days, were fractionated on a silicic acid column, and the specific activity of the free and ester cholesterol was determined. The total exchangeable cholesterol pool size was estimated from the extrapolated specific activity values for plasma cholesterol. All stool specimens were collected during each 10-day period, and the radioactivity was determined after fractionation. Plasma cholesterol was determined by a modification of the method of Sperry and Schoenheimer.

In general, the ester cholesterol pool approximated the free cholesterol pool in size, although the latter was usually smaller. A low-fat diet resulted in an increase in ester pool and a decrease in free pool in both instances, although plasma cholesterol fell in one patient and remained unchanged in the other. A high linoleic acid diet produced an increase in ester pool size in one patient, essentially no change in two patients, and decrease in one patient. Free pool size remained approximately the same in 3 patients and decreased in 1. In all 4 subjects there was a decrease in plasma cholesterol, averaging 21 per cent. Fecal excretion of radioactivity in the 10 days decreased in 3 patients.

The decrease in plasma cholesterol concentration without a consistent change in C^{14}-cholesterol pool size suggests that redistribution has occurred as a result of the dietary change.

67. THE ASSOCIATION OF PYRIDOXINE DEFICIENCY AND CONVULSIONS IN ALCOHOLISM

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(Introduced by Rudi Schmid, M.D.)

The alcoholic withdrawal syndrome includes convulsions. Some of these patients have well-documented idiopathic or posttraumatic epilepsy. Another group with convulsions, known colloquially as "rum fits," have seizures presumed to be due to alcohol withdrawal. In infants, adults, and laboratory animals, pyridoxine deficiency, produced either by malnutrition or by administration of isonicotinic acid hydrazide, is associated with grand mal seizures. An attempt, therefore, was made to determine whether patients with "rum fits" could have a similar pyridoxine deficiency.

Five patients with "rum fits," 3 patients with epilepsy and associated alcoholism, 5 patients in various phases of alcoholism without convulsions, and 1 normal nonalcoholic individual were studied. They were given a pyridoxine-free diet consisting of a partially hydrolyzed starch in water and were supplemented daily with vitamins excluding vitamin B_6. Ten grams DL-tryptophan was given orally, and the urine was collected for 24 hours. Following this, 100 mg. pyridoxine hydrochloride was injected intramuscularly; again, 10 Gm. tryptophan was given and urine collected for another 24 hours. Xanthurenic acid excretion for each of the two 24-hour tryptophan load tests was measured.

Highest excretions of xanthurenic acid occurred in patients with "rum fits." Significantly lower values were found in both control groups—epileptics with associated alcoholism and patients in various phases of alcoholism. Averaged values showed a fourfold increase in xanthurenic acid excretion in the patients with "rum fits" as compared with the control groups.

After 100 mg. pyridoxine intramuscularly, xanthurenic acid excretions decreased strikingly. The normal individual excreted no xanthurenic acid before or after pyridoxine administration.

Xanthurenic acid excretion after a tryptophan load is considered a reliable indication of pyridoxine deficiency. The findings in patients with "rum fits" that tryptophan loading resulted in the excretion of large amounts of xanthurenic acid and that correction of this metabolic defect occurred after the administration of pyridoxine suggest that "rum fits" are etiologically related to pyridoxine deficiency. A lesser degree of pyridoxine deficiency is probably responsible for the excretion of smaller amounts of xanthurenic acid in alcoholic patients without convulsions.

68. THE INFLUENCE OF WHOLE BODY IRRADIATION ON ERYTHROCYTE GLYCOLYSIS IN THE HUMAN PATIENT

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Erythrocyte glycolysis was determined in human patients with disseminated malignancies who received total body irradiation for therapeutic purposes. The dosage of irradiation was 150 to 200 r (measured in air) and was administered in a single exposure. Clinical and hematologic studies were performed and will be reported separately.

These in vitro observations demonstrated reductions of 30 to 50 per cent below control levels in erythrocyte glycolysis of cells removed from the patients within one hour following irradiation. Recovery from this acquired metabolic defect usually became apparent within 24 hours.

Sodium fluoride in a concentration of 10 mm. per liter usually produced a similar quantitative reduction of glycolysis in erythrocytes removed from nonirradiated patients; however, this chemical provoked no additional reduction of glycolysis in erythrocytes removed from patients an hour following radiation exposure.

These data suggest that irradiation of the human patient promptly produces a reversible metabolic defect which is reflected by reduction of erythrocyte glycolysis under in vitro conditions. This effect may be due to certain alterations of enzyme activity.

69. THE FINDING OF URINARY PROTEOSE IN CERTAIN RENAL DISEASES

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The isolation of a proteose from the urinary proteins in patients with active renal disease, not detectable in proteinurias of other etiologies, is reported. This proteose is characterized by the following properties: (1) not coagulated by heat; (2) precipitated by sulfosalicylic and trichloroacetic acids (the precipitates dissolved on heating and reappeared on cooling); (3) precipitated by full saturation with ammonium sulfate; (4) nondialyzable; and (5) biuret positive, giving a weak reaction. Electrophoretically its position is predominantly at alpha-1 extending almost to the alpha-2 position; it yields a strongly positive periodic acid-Schiff reaction.

A simple test based on the solubility of this proteose was employed in a survey of the incidence of proteosuria in the proteinurias of a variety of clinical classifications. Seventy patients were studied. The usual methods of clinical classification were supplemented by renal biopsy in 17 of these patients. Urinary proteose was present in 42 patients with acute and chronic glomerulonephritis, pyelonephritis, and nephropathies associated with diabetes. The universal proteosuria of this heterogeneous group stands in contrast to the absence of proteosuria in 9 "healed" acute glomerulonephritic patients (with histologic evidence of healing of inflammatory glomerular changes) and in 14 patients who, although comparable in degree of proteinuria, showed no evidence of proteosuria. Of these 10 were in congestive failure and 4 others had proteinuria, unrelated to primary renal disease.

Three patients with a clinical diagnosis of lupus erythematosus (whose renal biopsies showed glomerular changes consistent therewith) showed proteosuria with proteinuria. Two patients with the same diagnosis but with no evidence of renal involvement showed neither proteosuria nor proteinuria.

These preliminary studies indicate proteosuria, in contradistinction to proteinuria, may be correlated with active parenchymal renal inflammatory changes. The absence of proteosuria in the proteinurias studied, the pathogenesis of which is probably not correlated with parenchymal inflammation, suggests that while proteinuria may be transudative or exudative, proteosuria is clinically indicative of an exudative process.

70. THE MECHANISM REGULATING PULMONARY CAPILLARY BLOOD VOLUME DURING CHANGE IN POSITION

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The volume of blood in the pulmonary capillaries (V_c) can be calculated by measuring the diffusing capacity of the lungs for carbon monoxide (D_{LCO}) at 2 or more alveolar oxygen tensions (J. Appl. Physiol. 11: 290, 1957). We have made such measurements, using a rebreathing technique (Federation Proc. 11: 95, 1958) in 6 normal subjects while recumbent and during head-up tilt at 45 degrees. Changes in lung volume during tilting were controlled by incorporating helium in the rebreathing mixture and measuring helium dilution as well as CO absorption. D_{LCO} invariably fell on tilting. In 5 of 6 subjects the decrease in D_{LCO} was due chiefly to a decrease in V_c, averaging 18.8 per cent. To investigate the mechanism of this decrease, tilting was repeated in these subjects during the infusion of a rapidly acting ganglionic blocking agent (Arfonad). All 6 subjects, including the one whose V_c had not changed on tilting during the control study, now demonstrated a lower V_c on tilting. The average decrease from the recumbent position was 23.7 per cent, and the V_c when tilted during Arfonad administration was 24.2 per cent less than the V_c when tilted during the control period. In 5 of these subjects tilting was repeated a third time while norepinephrine was infused. No significant decrease of V_c occurred on tilting. When recumbent there was no significant increase in V_c during norepinephrine as compared with the control. These findings suggest that V_c is a function of venous return, or more directly of right ventricular output, since the normal response to tilting was exaggerated by ganglionic blockade that would encourage venous pooling, and is blocked by norepinephrine whose intense vasoconstriction would decrease venous pooling.

71. CONTENT OF ALDOSTERONE, CORTICOSTERONE, HYDROCORTISONE, AND CORTISONE IN ADRENAL TISSUE OF PATIENTS WITH PRIMARY ALDOSTERONISM

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We have defined primary aldosteronism as a syndrome produced by overproduction of aldosterone and associated with excretion of normal amounts of 17-hydroxycorticosteroids and 17-ketosteroids. Most of over 100 cases of which we are aware have exhibited elevated levels of urinary aldosterone. Several, otherwise typical, have had only borderline aldosterone values and yet have yielded adrenal adenomas. Thus, the possibility exists that another mineralocorticoid, as yet unidentified, may have caused the disease.

It is estimated that only 1 to 5 per cent of the aldosterone produced escapes in the urine as such. It was thought that a clearer picture might be obtained from a steroidal analysis of the surgically removed adrenals of patients with primary aldosteronism. Analyses for the various steroids have been performed upon the adrenal tissue of 9 patients with primary aldosteronism (7 tumors, 2 hyperplasia) and, for comparative purposes, of 4 patients with Cushing's syndrome (2 tumor, 2 hyperplasia) and 1 case of idiopathic edema. All adrenal tissue was either extracted or frozen within an hour after surgical removal. Separation, identification, and semiquantitation of the corticosteroids were carried out by standard physicochemical methods.

All of the 9 cases of primary aldosteronism gave very high values for aldosterone, 496 to 3,208 gamma per kilogram. The next highest was 133 gamma per kilogram in a case of Cushing's syndrome.

Corticosterone levels were very high in 2 of the 9 cases of aldosteronism. The remaining 7 gave values similar to those found in the other conditions. Wide variations, of similar range, in concentrations of hydrocortisone and cortisone were found within each group.

Although the only distinguishing feature in the adrenals of patients with primary aldosteronism consists of their very high content of aldosterone, no correlation exists between that and the level of urinary aldosterone, which may fluctuate widely.

These results suggest that in the presence of typical clinical manifestations of primary aldosteronism, borderline values for urinary aldosterone (1) do not eliminate this diagnosis and (2) do not necessarily imply excessive production of a mineralocorticoid other than aldosterone.

72. HYPERCALCIURIA IN WILSON'S DISEASE WITH DATA ON THE EFFECT OF DL-PENICILLAMINE ON THE URINARY EXCRETION OF CALCIUM AND COPPER

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(INTRODUCED BY MARSCHELLE H. POWER, PH.D.)

The urinary excretion of calcium was studied in 5 patients with Wilson's disease while they were given a diet containing 213 mg. of calcium and 2.93 mg. of copper per day. The diagnosis of Wilson's disease was based upon

neurologic findings, presence of Kayser-Fleischer rings, and elevated urinary excretion of copper and alpha amino nitrogen. One patient had bilateral nephrocalcinosis and another had a solitary renal stone.

Four of the five patients demonstrated hypercalciuria ranging from 210 to 404 mg. per day during a 3-day period. Except for one patient, the serum values for calcium, phosphorus, alkaline phosphatase, carbon dioxide combining power, and chloride were normal.

The effect of DL-penicillamine was then studied in 4 of the patients while on the same diet. Each patient was given 500 mg. of DL-penicillamine orally 3 times a day, 30 minutes before meals. In each instance, over a 3-day period, the urinary excretion of copper increased 9 to 17 times, while the daily urinary excretion of calcium increased slightly in 3 patients (by 35 to 70 mg.) and decreased in one (by 36 mg.). In two normal subjects, the administration of DL-penicillamine under the same conditions did not significantly alter the urinary calcium excretion, but increased the urinary excretion of copper 13 times in one subject and 19 times in the other.

DL-Penicillamine did not significantly alter the urinary pH, titratable acidity, total nitrogen, alpha amino nitrogen, ammonia nitrogen, or phosphorus in 2 patients and 2 normal subjects.

This hitherto undescribed finding of hypercalciuria may be another manifestation of the renal tubular dysfunction in Wilson's disease. It may account for the nephrocalcinosis and renal stone found in our patients and may be related to the osteomalacia occasionally reported with this syndrome. It is interesting that the patient with the least neurologic evidence of Wilson's disease had the lowest urinary excretion of calcium (116 to 125 mg. per day). The acute administration of DL-penicillamine caused a marked increase in the urinary excretion of copper in patients and normal subjects alike, but only slight changes in the urinary excretion of calcium.

73. THE TREATMENT OF POLYCYTHEMIA VERA WITH TRIETHYLENE MELAMINE—FIVE YEAR FOLLOW-UP

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Sixty-three patients were treated with triethylene melamine (TEM) over the past 5 years. There were 20 women and 43 men. Ages ranged from 23 to 75 years of age; three-fourths of the patients were over 55 and 0.8 per cent under 40 years of age. Twenty-six patients did not receive prior therapy. Nine previously had received radioactive phosphorus and had had 24 venesections. Details of prior therapy were not available in 4. Three regimens were used: TEM alone; TEM in conjunction with venesections; and TEM with miscellaneous agents (Myleran, Daraprim, and novobiacin). To date analysis has been completed in 17 patients who received 43 courses of various forms of therapy.

TEM was used in 13 courses, initial doses ranging from 3 to 15 mg. per week for 2 to 21 weeks. Complete remissions occurred in all patients within 2 to 21 weeks and lasted for 8.5 to 221.5 weeks. Four patients required maintenance therapy.

Eighteen courses consisted of administering TEM either following or concomitantly with venesection. Initial dosage of TEM in these patients ranged from 0.9 to 17 mg. per week, for 1 to 63.5 weeks. Remissions occurred within 1 to 65.7 weeks and were maintained for 8.3 to 197.8 weeks.

Initial hematocrits ranged from 51 to 73 mm. and during remission did not exceed 54 mm. in men and 48 mm. in women. Initial leukocyte counts ranged from 5.9 to 45.6 (thousands) per c.mm.[3]. During remission 12 out of

17 patients had leukocyte counts of less than 3,500 per c.mm.[3]. Two patients developed profound pancytopenia which reverted after discontinuation of therapy. Two patients developed nausea, and two others a maculopapular eczema. These side effects were not of sufficient intensity to discontinue therapy.

Triethylene melamine appears to be as effective as other forms of therapy for polycythemia vera.

74. COLLIMATION OF SCINTILLATION COUNTERS BY DIFFERENCE COUNTING

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Several new methods of studying organ function require the use of external counters to localize and quantitate isotopes within the body. The validity of such measurements depends in large part on accurate collimation of the counters which are employed. Isotopes such as Na^{24}, K^{42}, Fe^{59}, Co^{60}, Zn^{65}, As^{76}, Rb^{86}, and Au^{198} have gamma emissions with energy over 1.0 mev. Half of these rays will pass through each centimeter thickness of lead shielding without interaction. Therefore, bulky and heavy shields (and correspondingly long crystal to skin distances) are necessary to achieve good collimation. To meet this problem, the feasibility of collimating scintillation counters by difference counting was explored.

With this technique, successive counts are made with and without a lead shield placed between the crystal of the counter and the area under study. Radiation from outside the intermittently shielded area is unaffected by the change in the position of the shield and makes no contribution to the difference in the two counts. Therefore, the difference in the two counts is representative of the radioactivity of the shielded area. A gamma ray spectrometer is used so that only unattenuated gamma rays are counted. Although collimation can be obtained with a small movable shield alone, the sides of the crystal are partially shielded to increase the difference between the two counts and reduce statistical variation. Making use of the same principles, the aperture of a conventional collimating shield can be intermittently closed. This arrangement is readily adaptable to rapid automatic operation, difference counts being obtainable at intervals of less than one second using commercially available scalers. The lags in response typical of ratemeters are almost eliminated.

Several difference collimators suitable for studying myocardial radioactivity in man have been tested because of an interest in estimating coronary blood flow rate from the speed at which the myocardium takes up Rb^{86} from the blood. Results comparable to those previously obtained with a 350 pound conventional collimator can be obtained by difference counting with an automatically operated head weighing less than 40 pounds.

75. MYOCARDIAL EXTRACTION OF Rb^{86}

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(Introduced by Richard J. Bing, M.D.)

Coronary blood flow has been calculated from the myocardial uptake of Rb^{86} as measured by an external radiation detector. In order to avoid coronary sinus catheterization, myocardial extraction of Rb^{86} was assumed to be

47 per cent of the arterial concentration based upon observations in a group of dogs. Further evaluation of this assumption has been made by perfusion of isolated rabbit hearts (Langendorff preparation).

Immediately after sacrifice, the heart was removed and suspended in a constant temperature chamber at 38° C. A catheter was inserted into the aorta. In this manner the coronary arteries could be perfused at a variety of flow rates with oxygenated rabbit blood containing 0.5 μc of Rb^{86} per 100 ml. Cardiac contractions resumed shortly after perfusion began. Outflow from the heart was collected, measured, and its Rb^{86} content determined. Perfusion was continued for 20 minutes, a comparison being made between myocardial extraction at various flow rates, both early and late during the experiment. Extraction was significantly less at comparable flow rates in the latter part of the experiment. This finding was confirmed by the observation of progressively decreasing values for extraction when the rate of perfusion was kept constant throughout. When extraction values were plotted against flow, the slope was exponential with higher extraction values at slower flow rates. The average myocardial extraction of Rb^{86} at 3 ml. per minute was 46 per cent with a standard deviation of 6.5 per cent. At 1.5 ml. per minute, extraction rose to 52 per cent. It fell to 38 per cent at 6 ml. per minute and to 30.5 per cent at 9 ml. per minute.

In the determination of coronary blood flow with Rb^{86}, the principal sources of error are inaccurate measurement of myocardial Rb^{86} uptake by the external radiation detector and variation in the myocardial Rb^{86} extraction. Although the results of these rabbit experiments cannot be directly extended to larger animals or man, the data suggest that in comparable ranges a 50 per cent increase or decrease in flow rate would result in an extraction error of the order of 15 to 25 per cent.

76. POSSIBLE VARIATIONS IN ADRENOCORTICAL RESPONSIVENESS AMONG DIABETICS BASED ON LEUKOCYTE CHANGES FOLLOWING GLUCOSE ADMINISTRATION

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(Introduced by Max Miller, M.D.)

In a search for metabolic variants among patients with diabetes mellitus, intravenous glucose administration (25 Gm. in 50 ml.) has been postulated as a mild stress, and changes in absolute lymphocyte count (A.L.C.) and total leukocyte count (W.B.C.) have been determined over a subsequent 2-hour period. Saline control experiments (13) showed essentially no change in A.L.C. or W.B.C. Following glucose administration, all 13 nondiabetic subjects showed significant depressions of A.L.C. and W.B.C. at 30 minutes (mean changes of –30.7 and –22.5 per cent) with return to initial levels at 2 hours. Two types of response were observed in diabetic subjects. Ten tests in 6 of these subjects gave 30-minute mean changes in A.L.C. and W.B.C. of –4.1 and –1.7 per cent. The balance of the tests (19) in diabetic patients showed normal leukocyte depressions (mean changes at 30 minutes of –25.7 per cent for A.L.C. and –22.1 per cent for W.B.C.). In either the leukocyte response or nonresponse elicited by glucose administration, the A.L.C. and W.B.C. changes were found to be highly concordant.

That the leukocyte changes observed following glucose administration reflect a response of the adrenal cortex still remains to be satisfactorily established. Correlations between levels of adrenal cortical activity and numbers of circulating leukocytes of various types have been made by others. Intravenous administration of hydrocortisone in 8 tests gave a mean depression of W.B.C. at 30 minutes of –19.6 per cent with return to the initial level by 2 hours. The finding that reserpine consistently blocks the W.B.C. depression following glucose in normal and responding diabetic subjects (mean change of W.B.C. at 30 minutes of –3.0 per cent in 12 tests) is further evidence for pituitary-adrenocortical implication in the leukocyte changes observed. The single subject with adrenal insufficiency tested so far showed no leukocyte depression following glucose administration.

When the correlation between the W.B.C. changes following glucose administration and an adrenocortical response can be more firmly established, the procedure used here may suggest itself as a test of adrenocortical function more satisfactory than eosinophil counting, more convenient than determinations of A.L.C., and, in the study of diabetic patients, provide a metabolic classification inviting of further work.

77. THE HEPATIC BINDING OF I^{131}-LABELED INSULIN IN HUMAN SUBJECTS DURING A SINGLE TRANSHEPATIC CIRCULATION

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Evidence has appeared recently indicating that insulin has a direct hepatic effect, producing hypoglycemia not only by increasing peripheral utilization but also by decreasing the hepatic output of glucose. Other data from Poulsen's and our laboratory have indicated that the administration of exogenous insulin via the portal vein, thereby simulating the route of endogenous insulin, results in a greater hepatic effect and a smaller peripheral effect than insulin administered via a peripheral vein. Since endogenous insulin is secreted into the portal circulation and traverses the liver before becoming available to other tissues, the magnitude of the hepatic and peripheral effects may, in part, be dependent upon the amount of insulin bound to the liver during its initial transhepatic circulation.

This study was designed to determine the magnitude of the hepatic binding of I^{131}-labeled insulin in 15 human subjects during a single transhepatic circulation. At abdominal operation, 5 to 7 ml. of a solution containing 600 mg. of inulin and 50 μc (0.65 unit) of I^{131} insulin was injected intraportally. Ten seconds later a brachial arterial blood sample was collected over a 10- to 15-second period. The concentrations of inulin and I^{131} insulin per milliliter of blood were determined and the volumes of distribution of inulin and labeled insulin calculated. Twelve control studies were performed by injecting the inulin and labeled insulin solution into the antecubital vein and collecting a brachial artery blood sample 10 seconds later over a 10- to 15-second period.

The results indicate that 54 per cent (range 41 to 68 per cent) of the injected insulin was bound by the human liver in a single transhepatic circulation. In the 12 control studies the volumes of distribution of inulin (2,001 ml.) and labeled insulin (2,027 ml.) were identical. By contrast, after intraportal injection the volume of distribution of labeled insulin (5,817 ml.) was more than twice that of inulin (2,775 ml.) indicating that more than 50 per cent of the insulin was bound to the liver in a single circulation. These data support, but do not prove, the concept that endogenous insulin has a major hepatic effect.

78. NORMAL RENAL MAGNESIUM CLEARANCE AND THE EFFECT OF WATER LOADING, CHLOROTHIAZIDE, AND ETHANOL ON MAGNESIUM EXCRETION

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There is a paucity of data available on normal renal excretion of magnesium. Therefore, values were obtained for 24-hour total urinary magnesium, total and ultrafiltrable serum magnesium, and 24-hour endogenous creatinine clearance. In 35 normal men on general diets an average of 91.5 per cent of the calculated daily filtered load was retained with a range of from 83.3 to 96.9 per cent. Magnesium clearance (UV/P), corrected for the Gibbs-Donnan equilibrium by the constant 0.867, ranged from 6.24 to 25.37 ml. per minute with an average of 12.94.

During low magnesium intake and during magnesium repletion in alcoholics, urinary magnesium excretion drops precipitously, demonstrating renal conservation of the ion. Conversely, when magnesium is injected parenterally, large quantities may appear in the urine. Our studies were extended to determine renal handling of magnesium after water loading, chlorothiazide, ammonium chloride, and ethanol ingestion. The results in 9 normal men show that one day of water loading with urine flow up to 6,000 ml. has no effect on the glomerular filtration rate and no effect on renal magnesium clearance. In 4 normal subjects chlorothiazide increased the urinary magnesium as well as the sodium and potassium excretion, but it had no magnesiuretic effect in 3 others. Ammonium chloride given to 2 normal subjects in amounts of 4 Gm. daily did not increase excretion of magnesium nor glomerular filtration rate, in spite of modest increases in urine flow. When ethanol was given to 3 normal subjects in a single dose of 1 ml. per kilogram of urine, magnesium rose two- to threefold over 3 preceding control periods. The magnesiuretic effect did not appear dependent on ethanol-induced water diuresis.

We conclude from these studies that in normal subjects with average diet, magnesium equilibrium is maintained with excretion of from 4 to 16 per cent of filtered magnesium. Chlorothiazide often causes magnesiuresis, while water loading and ammonium chloride have no effect. Ethanol produces a marked increase in magnesium excretion in acute experiments; such an increased excretion may be the cause of magnesium depletion in chronic alcoholism.

79. INSULIN-LIKE ACTIVITY OF THE PLASMA AND PLASMA ELECTROPHORETIC FRACTIONS AFTER GLUCOSE ADMINISTRATION

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Insulin-like activity of plasma was studied in 3 patients during the oral glucose tolerance test. Insulin-like activity of the plasma was determined by the rat hemidiaphragm technique of Groen and Willebrands. Electrophoretic separation was carried out on paper. Normal plasma showed no activity in the fasting sample, but activities were estimated per milliliter of plasma as follows: first hour, 0.72 mu.; second hour, 0.80 mu.; third hour, 0.26 mu.; fourth hour, 0 ($P < 0.05$). Electrophoretic fractions showed no activity in the fasting

sample, but activity in the alpha-2 and beta fractions in the subsequent samples with the peak in the beta fraction. Activity recovered from the electrophoretic fractions often exceeded activity of the intact plasma. Activity appeared first in the alpha-2 fraction but reached a maximum peak in the beta fraction at 3 hours.

Plasma from a patient showing a hypoglycemic response to the glucose tolerance test showed no activity. Electrophoretic fractions showed significant activity in the region of alpha-2 as follows: fasting, 0; first hour, 1.20 mu.; third hour, 0.51 mu.; fourth hour, 1.31 mu. ($P < 0.05$). No insulin-like activity was found in the beta fraction; in fact, a significant depressant activity was exerted by the beta fraction on the diaphragm.

Plasma from the young diabetic patient not treated with insulin showed activity in the fasting sample and in the second hour sample (0.61 and 0.68 mu., respectively). Activity was noted in the alpha-2 and beta fractions with the peak in the beta fraction. At the third hour the recovery from the fractions exceeded that for the whole plasma.

This interpretation of plasma insulin levels should take into consideration the facts that activity may be found in the electrophoretic fractions and not in the whole plasma and that activity is greatly altered by ingestion of glucose.

The predominate location of activity in the alpha-2 region in the hypoglycemic patient is consistent with the hypothesis that insulin is in the plasma in the free state, since this is at the electrophoretic location for free insulin.

80. THE EFFECT OF THE BLOOD GLUCOSE ON INSULIN SECRETION

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(Introduced by A. R. Colwell, Sr., M.D.)

The evidence at present available concerning insulin secretion permits only the limited conclusion that hyperglycemia produces an increase in insulin output. The purpose of the present experiments was to explore the quantitative relationship between blood glucose values, both high and low, and the resulting insulin secretion.

The venous effluent from the body and head of the pancreas was continuously collected in anesthetized heparinized dogs by means of a plastic cannula secured in the pancreatic vein. Samples of this blood were assayed for insulin content by means of the isolated rat diaphragm technique. Assays were performed in duplicate on 35 samples obtained from 10 dogs. The flow rate through the cannula, which in each case had a diameter larger than that of the respective pancreatic vein, was measured by collecting the blood into graduated tubes. Low blood glucose values were produced in certain dogs by the administration of phlorhizin for 3 days preoperatively. High values were obtained by the infusion of glucose into the femoral vein during the experimental procedure.

The results demonstrate that insulin concentration in the pancreatic venous blood is a function of the blood glucose concentration. With blood glucose values in the normal range of 91 to 170 mg. per cent, the mean insulin concentration in the pancreatic venous blood was 75 mu. (milliunit = 0.001 unit) per 100 ml. serum. In the hypoglycemic range of 35 to 90 mg. per cent, the mean insulin concentration was depressed to 20 mu. per 100 ml., about the same as that found in the peripheral blood in the normal fasting animal. In the hyperglycemic ranges of 171 to 350 and 351 to 700 mg. per cent, the mean

insulin concentrations were markedly elevated to 250 and 540 mu., respectively. The form of the relationship between the insulin concentration and the blood glucose concentration is curvilinear with the concavity upward and is rectified by a logarithmic transform of both variables. Interpolation on the curve indicates that a two and one-half fold increase in insulin concentration occurs when the blood glucose rises from 100 to 200 mg. per cent.

81. A COMPARATIVE STUDY OF SOME RECENT SEROLOGIC TESTS FOR RHEUMATOID ARTHRITIS

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(Introduced by Franklin D. Johnston, M.D.)

One of the important recent developments in the diagnosis and study of rheumatoid arthritis has been the introduction of a number of serologic tests which it is hoped will prove to be specific. In general, these procedures depend upon the reaction between a so-called "rheumatoid factor" in the patients' sera and gamma globulin from pooled human sera. Most of the tests are based on agglutination of an indicator particle (such as the sheep erythrocyte, latex, or Bentonite particle) which has previously been coated with gamma globulin.

The present study was undertaken in order to compare the performance of the Bentonite flocculation and latex-fixation tests with the Ziff modification of the sensitized sheep cell test using divided specimens of sera from patients with definite rheumatoid arthritis, a variety of other rheumatic diseases, and nonrheumatic controls. As a background, over 2,000 sera have been tested by the Ziff procedure in our laboratories; in this large series the incidence of positive results in 327 patients with definite rheumatoid arthritis was approximately 76 per cent. In the comparative study, positive results were obtained with sera from 140 patients with definite rheumatoid arthritis in 58.5 per cent of instances with the Bentonite procedure, 64 per cent with the latex test, and 75.5 per cent with the sensitized sheep cell test. In 52 nonrheumatoid controls the incidence of positive results with the 3 procedures was 2, 2, and 11.5 per cent, respectively. It would appear that both the latex-fixation and Bentonite tests may be more specific although somewhat less sensitive than the sensitized sheep cell test.

The relative merits of the test procedures will be briefly discussed, along with the practical difficulties in interpretation of test results.

82. DIFFERENTIAL ADSORPTION OF HORMONES FROM SERUM: A NEW PROCEDURE

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(Introduced by J. M. Hayman, Jr., M.D.)

The difficulties inherent in the traditional methods of measuring alterations in serum binding of hormones have prompted the development of a new technique. An in vitro system was designed which quantitated the differential adsorption of labeled hormones from serum by a resin, using L-thyroxine or insulin as representative hormones. Sera containing hormones, labeled with I^{131}, were equilibrated for 1.5 hours in glass tubes with a measured volume

of Amberlite IRA-400 anion exchange resin. Following equilibration the tubes were counted in a conventional well-type scintillation detector, and the resin was then washed to remove the serum-bound radioactivity. The activity remaining on the resin divided by the initial activity measured the fraction of hormone bound by the resin ("resin index"). Thus a low resin index reflected increased binding of hormone by serum and vice versa. The mean difference between duplicate resin indices of 276 sera was 1.2 ± 1.0 per cent (1 S.D.).

Since alterations in hormone binding had been demonstrated by electrophoretic techniques in insulin-treated diabetes, thyroid disease, and pregnancy, sera from these conditions were selected for study. Increased binding of I^{131} insulin by the sera of 35 previously treated diabetic patients was shown by a decreased index (11.9 ± 5.6 per cent) when compared with 57 control sera (25.6 ± 2.6 per cent, $P < 0.001$). The resin indices for I^{131} thyroxine were distinctly higher in 10 hyperthyroid (58.6 ± 7.1 per cent) and lower in 12 hypothyroid subjects (39.6 ± 4.6 per cent) when compared with 20 euthyroid control subjects (47.8 ± 2.7 per cent, $P < 0.001$), indicating increased serum binding of hormone in myxedema and decreased in hyperthyroidism. Standardized resin indices using I^{131} thyroxine were not obtained for the pregnancy group because of variations in indices observed with different batches of resin. However, 70 pregnancy sera bound more thyroxine than did 75 nonpregnancy sera in the same batches, with overlap of only a single resin index in one control subject.

In summary, quantitative alterations in serum-hormone interactions have been demonstrated in treated diabetes, pregnancy, and thyroid disease, using differential adsorption of hormones by a resin.

83. INVESTIGATIONS OF THE SITE OF ACTION OF THE THYROID HORMONE: STUDIES IN HUMAN ERYTHROCYTES

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Although it has long been known that thyroid hormone stimulates oxygen consumption, the mode of action of thyroxine and its derivatives on cells is poorly understood. Increased oxygen consumption without increased high energy phosphate bond formation has been observed in vitro in many tissues, but location of the site of this metabolic effect has been hampered by the multiplicity of metabolic pathways in most cells. Glucose is oxidized without concurrent formation of high-energy phosphate bonds in the hexose monophosphate (HMP) pathway. This route of metabolism may thus be a possible point of stimulation. The human erythrocyte is an ideal system in which to study oxygen consumption via this pathway, since other pathways of oxygen consumption are virtually absent.

Triiodothyronine (TRIT), a metabolically active thyroxine analogue, stimulated oxygen consumption of red blood cells. To confirm the fact that this stimulation occurred along the HMP pathway, production of $C^{14}O_2$ from glucose-1-C^{14} and glucose-6-C^{14} was compared. $C^{14}O_2$ was produced almost exclusively from the 1-carbon atom of glucose. This finding is consistent with that expected if decarboxylation occurred in the HMP pathway. In order to determine whether increased metabolism could be explained on the basis of changes in membrane permeability, the experiments were repeated using hemolyzates produced by freezing and thawing. Again, stimulation of oxygen consumption by TRIT was demonstrated. Stimulation could not be achieved

with metabolically inactive substances such as diiodotyrosine, dinitrotyrosine, or iodide even in concentrations 20 times as great as stimulating concentrations of TRIT.

An attempt has also been made to determine the precise point at which TRIT stimulates metabolism through the HMP pathway. The following observations have been made: (1) TRIT is not a substitute for TPN; (2) TRIT does not stimulate glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase activity; and (3) TRIT stimulates enzymatic oxidation of TPNH in a system consisting of TPNH, methylene blue, and methemoglobin reductase (TPNH-oxidase), prepared by the purification procedure of Huennekens. These observations are interpreted as indicating that in the mature human erythrocyte, TRIT increases oxidative metabolism of glucose via the HMP pathway by stimulating the oxidation of TPNH.

84. DIAGNOSTIC USEFULNESS OF THE LEFT ATRIAL PRESSURE PULSE IN RHEUMATIC MITRAL VALVULAR DISEASE

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(INTRODUCED BY LOUIS N. KATZ, M.D.)

The significance of left atrial pressure pulse contour as an index of rheumatic mitral stenosis and/or insufficiency was evaluated by several available methods, using 51 tracings from 41 patients. Thirty-eight pressures were recorded directly at surgery and 13 at left heart catheterization. The cases were divided into 3 groups according to evaluation of the valve at surgery: (1) "pure" mitral stenosis, (2) significant mitral stenosis with definite mitral insufficiency, and (3) pure or predominating mitral insufficiency.

No separation of the curves into the above groups was obtained by methods which included those of Kent and associates. Calculations involving the rate of the Y descent presented insuperable difficulties due to variations in heart rate and inability to accurately determine an end point. A definite isotonic line, supposedly indicative of mitral insufficiency, was found in two severe cases of mitral stenosis. Diastasis was of no diagnostic value.

A remarkably good separation of Groups 1 and 3 was obtained with a modified RY/V ratio, using the rate of Y descent only during its first 0.10 second. This calculation was simple and easily reproducible. There was no advantage in relating this portion of the Y descent to mean left atrial pressure as suggested by Morrow. There were no false negative results with regard to mitral insufficiency in Group 3, but 5 cases in Group 1 gave false positive values. Each of the latter had evidence of an associated aortic valvular lesion and/or left ventricular failure. The results were equivocal in Group 2. Calculations by this method using surgical pressure pulses did not differ materially from those obtained during left heart catheterization of the same patient. Systolic washout of the catheter from the left ventricle during left heart catheterization was a prominent feature in Groups 2 and 3.

85. EFFECTS OF AMINE LIBERATORS ON GASTRIC SECRETIONS IN THE RAT

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(INTRODUCED BY A. C. ERNSTENE, M.D.)

Acute hemorrhagic ulcers restricted to the glandular portion of the stomach can be readily elicited in rats by endogenous liberation of histamine and/or serotonin. Whether the amines involved act by increasing gastric acid

secretions is the object of the present paper. This was investigated in groups of at least 6 adult rats each, in which the pyloris was ligated following fasting. Drugs were then injected; the animals were deprived of fluid and killed 6 hours later. Volume, pH, and free and total acidities were measured. In the controls the following mean values were obtained: volume 12.6 ml., pH 1.2, free acidity 71 degrees, and total acidity 113 degrees. With histamine liberators (polymyxin B, compound 48/80, dextran, or ovomucoid) there were decreases in volume and acidity independent of the presence of ulcers. Thus, with polymyxin B (1 mg. per kilogram) the respective values were 5.4 and 2.4 ml., 21 and 68 degrees. When promethazine (50 mg. per kilogram) was given with polymyxin B, volume and acidity were further depressed and ulcerations were prevented. With atropine (5 mg. per kilogram) plus polymyxin B the volume was reduced to 1.9 ml. with no detectable acidity, while ulcers were present in all the rats. The effects of 5-hydroxytryptophan (300 mg. per kilogram) were in the same direction, but not as marked as those with histamine liberators. The respective mean values were 6.3 and 1.5 ml., 54 and 115 degrees; these were not significantly altered by simultaneous treatment with BAL (4 mg. per kilogram), although ulcers were inhibited. Injections of histamine (30 mg. free base) and/or serotonin in oil (6 mg. free base) also reduced volume and free acidity; no ulcer was present, although serotonin produced severe renal cortical necrosis. In conclusion, amine liberators do not produce ulcerations in rats as a result of an increase in volume and acidity of gastric secretions. Probably their mechanism of action is primarily due to the effect of amines on the vessels of the stomach.

86. IODIDE GOITER

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Four women and one man receiving protracted therapy with iodides for asthma or bronchitis with diffuse thyroidal enlargement were studied. Needle biopsy of the thyroid gland revealed parenchymatous hypertrophy in all; 2 were euthyroid and 3 were hypothyroid. Tracer studies with I^{131} showed increased thyroidal clearance while iodides were being used, whereas the 6-hour and 24-hour thyroidal uptakes were increased only after the use of iodides had been discontinued for more than 24 hours. When one myxedematous patient ceased to use iodides clinical evidence of myxedema and the goiter disappeared within 3 months. Results of tracer studies with I^{131} showed very high 2-hour, 6-hour, and 24-hour thyroidal uptakes of the element which were inhibited markedly by administration of 2 mg. of potassium iodide during a subsequent tracer study. In the other patients the goiter and myxedema disappeared after administration of desiccated thyroid.

One euthyroid patient was observed for 2 years; in this patient results of studies of I^{131} kinetics were more thoroughly evaluated than in the others. Nine days after withdrawal of iodides the 24-hour thyroidal uptake of I^{131} was 92 per cent. The conversion ratio at 48 hours was 100 per cent and the protein-bound iodine^{131} was shown by chromatography to be thyroxine. The need for iodides continued. Therapy with desiccated thyroid was instituted, and 5 months later the goiter had disappeared. Discontinuation of use of desiccated thyroid resulted in reappearance of the goiter and mild myxedema 5 weeks later. Administration of iodides was then stopped; 20 hours afterward the thyroidal clearance of I^{131} was 74 ml. per minute with a peak uptake of 25 per cent (uncorrected) at 50 minutes and a steady decrease thereafter. Disappearance rates of I^{131} from the thyroid and thigh were 6.40 and 6.12

per cent, respectively, and the appearance rate in the urine was 6.19 per cent per hour. The value for I^{127} in the serum was 12.4 gamma per 100 ml. These studies were repeated 24 hours later with essentially similar results. Another biopsy of the thyroid gland was done for radioautography and assessment of iodine binding.

87. INFECTION PATTERNS IN PERMANENT STRAINS OF HUMAN CELLS

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The "microepidemiology" of vaccinial infection has been studied in 3 permanent lines of human cells. The course of infection is characteristic for each cell line and appears to be related primarily to the physical relationships between the cells.

The cells are propagated on glass. Cells of strain HeLa (Gey) are epithelial-like and tend to remain attached and form sheets. Strain FS14 retains the morphologic characteristics of "normal" fibroblasts. The cells tend to remain attached by long processes. Fibroblasts of strain U12 have undergone morphologic transformation. Contact between the cells is minimal until crowding occurs since they separate after division and migrate freely.

Vaccinial infection of FS14 fibroblasts leads to destruction of the culture regardless of cell numbers. The course of infection in U12 fibroblasts differs markedly. If most of the cells are separated, individual fibroblasts and small groups are infected. With regular feeding, the cells increase in number to form a moderately compact sheet. Then virus activity increases dramatically to involve most of the cells. A few isolated cells remain which repopulate the flask. Infected cells can always be found and virus is continually present. Ultimately, the population is comparable to that at which the first "epidemic" occurred; infection spreads rapidly, and again the culture is nearly destroyed. Such "epidemic cycles" have been observed for many months and thus far there is no evidence of development of resistance in the cells. Infection in the HeLa cultures exhibits characteristics of both fibroblast systems. Infection often begins in a single cell of a colony and extends to the others. Frequently, separated cells escape to establish clones which in turn are infected. The prospect of a cell contracting infection is much greater than in the U12 system, and thus the probability of destruction of the culture is higher.

The implication of these findings will be discussed with regard to the physiology and culture characteristics of cells in vitro and to the pathogenesis of vaccinial lesions.

88. QUANTITATIVE MEASUREMENT OF ALKALINE PHOSPHATASE AND LACTIC DEHYDROGENASE ACTIVITIES IN THE ANATOMIC UNITS OF THE HUMAN NEPHRON

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The purpose of this study is to investigate the role played by enzymes in urine formation by nephrons of normal and diseased kidneys, with particular reference to tubular reabsorption of glucose.

Renal tissue obtained by biopsy was frozen rapidly in liquid nitrogen and sectioned. The anatomic units of the nephron were identified accurately in stained sections, and the adjacent unstained lyophilized sections were dissected out with microscalpels and a dissection microscope. The minute fragments of glomeruli and tubules were handled with micromanipulators and accurately weighed on a quartz fiber balance (sensitivity 4×10^{-10} grams). Sample weights ranged from 10^{-8} to 10^{-7} grams, corresponding to 20 to 200 cells. Thereafter, the tissues were transferred to the bottoms of microtest tubes for incubation, and enzyme activities were determined quantitatively by ultramicrobiochemical techniques (Science 127: 1342, 1958).

Because alkaline phosphatase has been considered to be concerned in tubular reabsorption of glucose and, because Stowers and Dent observed decreased stainable alkaline phosphatase in Fanconi's syndrome kidney; its activity was determined quantitatively in kidney specimens from 6 healthy adults and patients with Fanconi's syndrome, familial renal glycosuria hypophosphatasia, cystine storage disease, systemic lupus erythematosus, and rheumatoid arthritis. Lactic dehydrogenase activity was also studied. These enzymes were also determined in renal biopsies from dogs before and after glycosuria was produced by phlorhizin.

The data indicate the following. 1. That enzyme activity can be measured quantitatively in individual functional units of the nephron.

2. A parallel decrease in activities of both enzymes was observed only in Fanconi's syndrome, suggesting that these changes reflected a specific effect of the disease.

3. Changes in enzyme activity were observed in the absence of local histologic abnormality, suggesting that the biochemical changes might be earlier and more sensitive indices of damage.

4. Alkaline phosphatase was decreased or virtually absent in proximal tubular cells of some patients with *no* glycosuria and is, therefore, not a key enzyme in glucose reabsorption in man. This was confirmed by the phlorhizin experiments in dogs, there being no demonstrable difference in enzyme activity before and after glycosuria appeared.

89. OCCULT INTRAVASCULAR CLOTTING

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Acquired hypofibrinogenemia may result from premature separation of the placenta, retention of a dead fetus, cancer of the prostate, and other causes. The mechanism whereby the depletion of fibrinogen is brought about is not known. An attempt has been made to produce experimentally a condition simulating the clinical state. For this purpose, the injection of a dilute thrombin solution intravenously was employed. To avoid massive intravascular clotting, the concentration and rate of injection were carefully standardized. The maximum dose that could be given with safety to a dog was found to be 70 units per kilogram of body weight injected at a constant rate per hour. As was to be expected, and confirmatory of the findings of previous investigators, the concentration of fibrinogen steadily decreased and, likewise, the platelet count fell. More significant, however, the clotting time was strikingly increased and occasionally was difficult to determine because of the poorly organized clot. The prothrombin time in some experiments increased from a normal of 6 seconds to over 2 minutes. This was found to be caused by a marked diminution of labile factor (factor V) and to a lesser degree by loss of prothrombin. The stable factor (factor VII) concentration was not found

to be altered. The prothrombin consumption was, likewise, greatly diminished and this was principally due to a decrease of thromboplastinogen (factor VII). No decrease of PTC (plasma thromboplastin component, factor IX) was found. When the injection of thrombin was continued for several hours, a tendency to bleed profusely from venipuncture was observed, and a marked hemolysis was also noted. The results of this study demonstrate that a state of hypocoagulability is induced in dogs by the intravenous injection of thrombin. No massive thrombosis occurs, but the fibrinogen is gradually diminished. It appears probable that the chief cause of the hypocoagulability is the drastic reduction of labile factor and thromboplastinogen. These findings suggest that perhaps the defective hemostasis in acquired clinical hypofibrinogenemia may not be due primarily to lack of fibrinogen but to the loss of other essential clotting factors.

90. EFFECT OF REMOVAL OF *N*-ACETYLNEURAMINIC ACID FROM ERYTHROPOIETIN

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We have recently reported the isolation of erythropoietin from acidified, boiled filtrates of anemic rabbit plasma by DEAE-cellulose ion-exchange techniques, and have shown that this active principle is a low molecular weight acidic glycoprotein. In the work to be reported erythropoietin has been submitted to mild acid hydrolysis and the hydrolytic products studied by biologic and chromatographic methods.

Hydrolysis of erythropoietin in 0.01 N sulfuric acid removes all of its *N*-acetylneuraminic acid (sialic acid) content and reduces its paper anodic mobility to a minimum value of 25 per cent of normal. Paper chromatographic analysis of the hydrolysis products obtained with 0.04 or 0.1 N H_2SO_4 reveal erythropoietin to contain N-acetylneuraminic acid, glucosamine, fucose, galactose, and mannose.

Comparison in the rat of the erythropoietic activity of dialyzed hydrolyzate of erythropoietin, obtained with 0.01 N H_2SO_4, and intact erythropoietin by use of reticulocyte counts and the 24-hour uptake of Fe^{59} by the total red cell mass shows that erythropoietin from which all neuraminic acid has been removed is not active.

In further studies we have passed whole plasma from phenylhydrazine anemic rabbits through DEAE-cellulose columns. This procedure removes a low molecular weight acidic glycoprotein from the plasma. This substance has a biologic activity and a chemical structure similar to that of erythropoietin isolated from boiled plasma filtrates. Additional studies on this glycoprotein will be presented.

91. SPECIFIC ECOLOGY AND CHRONIC ILLNESS

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If a person is adapted to a common food or some other oft-repeated environmental substance to which susceptibility exists, each regular exposure is followed by an immediate "pickup." Such an adapted person tends to arrange the frequency and size of re-exposures so as to remain continually "picked up." Or, stated in reverse, he resorts to a "pick-me-up" as often as necessary to avoid the onset of "hang-overs" as well as to dispell those

already present. This thrust and parry of specific adaptation characterizes a large segment of human ecology—the science dealing with the mutual relations between man and his environment.

But, if susceptibility increases, if adaptation to specific heterogeneous materials tapers off, or if the number of offenders becomes burdensome, "pick-ups" become shorter and less effective and "hang-overs" predominate. Chronic respiratory, dermatologic, gastrointestinal, and other localized withdrawal effects emerge as the "onset of the present illness." With further advancement, one or more of these manifestations may be associated with, may alternate with, or may be superseded by chronic fatigue, headache, myalgia, arthralgia, arthritis, mental, neurologic, and/or alleged psychosomatic syndromes. These maladapted chronic reactions are usually characterized by numerous complaints and are sometimes complicated by obesity or alcoholism.

Although chronic symptoms may be accentuated initially, they are usually relieved as a result of comprehensive environmental control. Drugs, cosmetics, and tobacco are avoided while fasting on spring water in hospital quarters freed of air-borne particles and fumes. This program induces nonadapted states in which immediate diagnostic test reactions are precipitated when susceptible individuals are re-exposed. The effects of isolated doses of single foods and of foods contaminated by insecticides, fumigants, preservatives, and sulfur or packaging materials, or those artificially colored, sweetened, flavored, or ripened are determined. Cumulative test doses may be necessary if specific susceptibility is low. Demonstrated offenders are avoided as incriminated. Then maintaining a compatible intake, patients are observed upon returning to their cars, homes, water supplies, work, avocations, and other avoided exposures.

The ecology of patients manifesting chronic illness of unknown or nondemonstrable origin should be investigated by means of comprehensive environmental control followed by isolated spaced re-exposures.

92. THE INFLUENCE OF ATRIAL AND VENTRICULAR SEPTAL DEFECTS ON THE CAPILLARY BED OF THE LUNG AND ON THE DIFFUSION CHARACTERISTICS OF THE PULMONARY MEMBRANE

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It has been shown previously that the diffusing capacity of the lung for carbon monoxide is dependent not only on the physical properties of the alveolar capillary membrane but to a comparable extent depends on the pulmonary capillary blood volume and on the rate at which erythrocytes within the pulmonary capillary bed take up CO. If the diffusing capacity of the lung for carbon monoxide (D_{LCO}) and the in vitro rate of combination of CO with intracorpuscular hemoglobin (O) are known for several different oxygen tensions, it is possible to assess the relative importance of these factors and to determine the "true" diffusing capacity of the pulmonary membrane (D_M) and the average pulmonary capillary blood volume (V_C). Measurements of pulmonary hemodynamics and of diffusing capacity (at oxygen tensions between 100 and 600 mm. Hg) were made on 12 patients with atrial or ventricular septal defects to determine the effect of variations in pulmonary blood flow on the size of the capillary bed of the lung and on the diffusing capacity of the pulmonary membrane. In the patients studied, pulmonary blood flow varied from 2.6 to 24.2 liters per minute and mean pulmonary artery pressure

from 16 to 80 mm. Hg. As expected, pulmonary diffusing capacity was influenced significantly by variations in pulmonary blood flow and in the volume of blood within the capillary bed. In the absence of pulmonary hypertension D_L was increased 11 to 92 per cent due to a proportionate increase in D_M and V_C occasioned by the increased pulmonary blood flow. In those patients who had developed pulmonary hypertension and in whom pulmonary arteriolar resistance had increased significantly, D_L was decreased. In 3 patients the decrease in D_L was due to a disproportionate reduction in D_M. In these the ventricular end diastolic pressures exceeded 13 mm. Hg. Thus, pulmonary diffusing capacity should be increased in the presence of an increased pulmonary blood flow. In patients with atrial or ventricular septal defects a reduction in pulmonary diffusing capacity probably is an indication of the presence of pulmonary hypertension or incipient cardiac failure.

93. THE SURVIVAL OF AUTOTRANSFUSED Cr^{51}-LABELED ERYTHROCYTES IN INFANTS WITH SEVERE IRON DEFICIENCY ANEMIA

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The survial of autotransfused Cr^{51}-labeled erythrocytes was determined in 14 iron-deficient infants 7 to 22 months of age. A similar study has been performed on 5 hematologically normal infants ages 7 to 30 months. Iron deficiency was documented by the following findings: hemoglobin, 1.8 to 7.3 Gm. per 100 ml.; MCHC, 19 to 28 per cent; MCV, 50 to 80 cu. microns; saturation of iron-binding protein, 0 to 15.4 per cent; and typical response to intramuscular iron therapy.

The erythrocyte "half-life" of the anemic patients ranged from 13 to 22 days, the average being 17 days. The erythrocyte "half-life" of the control group ranged from 28 to 35 days, the average being 32 days. Neither erythrocyte autosurvival nor significant reduction of red cell half-life in iron-deficient infants has been reported heretofore.

Blood loss through the gastrointestinal tract was investigated as a possible explanation for the short "half-life" of the erythrocytes in 11 of the anemic infants. Daily urine-free stool specimens were collected in the metabolic unit. Each specimen was tested for occult blood and then was put into a 4-day pool that was assayed for radioactivity to quantitate gastrointestinal bleeding. All subjects had stools intermittently or continuously positive for occult blood; 66 per cent of the stools collected gave a positive test. Estimated blood loss ranged from 0.25 to 3.7 ml. per day. Blood loss of this amount is not of sufficient magnitude to account for the observed decreased erythrocyte survival times. However, this evidence for gastrointestinal blood loss suggests that small loss over an extended period of time may play a role in the etiology of iron deficiency anemia in infants.

94. MECHANISM OF NOREPINEPHRINE-INDUCED PULMONARY CONGESTION IN AORTIC INSUFFICIENCY

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The influence of the sympathetic nervous system upon cardiovascular dynamics in normal subjects and in patients with aortic regurgitation has

been simulated by the infusion of norepinephrine (0.2 γ per kilogram per minute for 15 min.). Simultaneous with similar increases in arterial pressure in both groups, the most striking response in the 7 patients with isolated aortic valve insufficiency and compensated hearts was an elevation of the pulmonary "capillary" pressure from 9 to 30 mm. Hg ($p = <0.001$) in contrast to a 5 mm. rise in 5 normal subjects. Whereas there was a slight decrease in heart rate and cardiac output in normal subjects (6.4 to 5.9 L. per min.), the aortic insufficiency group had no significant change in rate and considerable depression of total cardiac output (8.74 to 6.74 L. per min.; $p = <0.01$). While the forward flow (Hamilton's method) fell from 5.14 to 4.70 L. per min. ($p = <0.05$), the regurgitant flow (Korner-Shillingford, downslope of indicator dilution curve) fell from 3.51 to 1.54 L. per min. ($p = <0.01$). This represented a change from 66 per cent to 40 per cent of forward flow.

To test the postulate that the elevated hydrostatic pressure in the pulmonary capillary represented pulmonary congestion due to blood displacement from the periphery, 3 patients received norepinephrine after tourniquets were applied on 3 extremities. Despite the usual arterial pressure increase, the pulmonary capillary pressure did not rise above normal. Mean regurgitant flow increased from 3.17 to 4.01 L. per min., while forward flow diminished slightly from 5.25 to 5.13 L. per min.

Thus, the hemodynamic response to norepinephrine in aortic insufficiency appears to depend upon displacement of blood to the pulmonary vascular bed and to the grossly enlarged heart unable to respond to elevation of filling pressures with an increase in cardiac output. The paradoxical decrease in regurgitant flow in the face of elevated arterial pressures may be largely explained on the reduced aortic-ventricular diastolic pressure gradient and diminished forward flow.

95. OBSERVATIONS ON THE TUBULAR REABSORPTION OF PHOSPHATE IN THE DIFFERENTIAL DIAGNOSIS OF HYPERCALCEMIA AND HYPOPHOSPHATEMIA

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(INTRODUCED BY WILLIAM H. DAUGHADAY, M.D.)

The tubular reabsorption of phosphate (TRP) was measured in 60 subjects in whom abnormalities of calcium and phosphorus metabolism were suspected. The results indicate that the TRP is of distinct but limited value in the differential diagnosis of hypercalcemia.

TRP was calculated from the simultaneously measured clearances of endogenous creatinine and phosphate under standard fasting conditions. Sixty subjects were studied and classified as follows: 8 control subjects; 23 cases of renal calculi; 2 cases of idiopathic hypercalciuria; 5 cases of parathyroid adenoma; 6 cases of hypercalcemia associated with cancer; 1 sarcoid case; 4 cases of osteomalacia; 2 cases of renal tubular acidosis; and 9 miscellaneous patients.

The TRP, expressed as per cent of filtered phosphate reabsorbed, was 85.5 ± 5.4 (mean $\pm$ 1 S.D.) in normal subjects and 85.0 ± 3.2 in euparathyroid patients with renal calculi. These values do not differ significantly, and the 95 per cent confidence limits for these combined groups were 77.3 to 92.9 per cent. In the hyperparathyroid patients, the mean TRP was 60.9 per cent and

the range of values was 55.0 to 68.6 per cent. The TRP was decreased in all 6 of the patients with malignancies and hypercalcemia but was decreased into the range observed in hyperparathyroidism in only 2 instances. The patient with sarcoid and hypercalcemia had an unusually low TRP of 48.1 per cent. The TRP was normal in the patients with renal tubular acidosis and normal or only slightly decreased in osteomalacia.

Most subjects were studied with a dietary history of unrestricted food intake. The importance of an adequate intake of phosphorus preceding measurement of the TRP is strongly emphasized; a restricted intake of phosphorus results in very high values of the TRP in normal subjects and, in hyperparathyroid patients, phosphorus deprivation results in an increase of the TRP from very low values to the normal range.

The difficulty in the differentiation between occult malignancy with hypercalcemia and primary hyperparathyroidism is lessened slightly by the TRP; very low values suggest hyperparathyroidism but do not exclude malignancy. The TRP differentiates clearly between osteomalacia and hyperparathyroidism not associated with hypercalcemia. Its principal usefulness appears to lie in determining the cause of hypophosphatemia and as a screening procedure for patients with renal calculi and borderline abnormalities of serum calcium and phosphorus.

96. THE HEART AS A CONTRACTING SPHERE

Geometric Relations Among Myocardial Tension, Oxygen Consumption, Coronary Blood Flow, Efficiency, Cycle Length, Duration of Systole, and Heart Size

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When the heart is viewed as a contracting sphere, certain properties of this pump may be explained, since the tension generated in the myocardium during systole can be shown to be related directly with cardiac oxygen consumption and coronary blood flow. For these studies we have obtained continuous direct measurements of total coronary flow and arteriovenous oxygen differences in an innervated, metabolically normal preparation (J. Appl. Physiol. 6: 311, 1953), as variations were induced in cardiac outputs, arterial pressures, and heart rates. Calculations were based on the assumption that the heart was a sphere with an internal radius (r) proportional to the cube root of the stroke output. Tension was calculated as $\frac{1}{2}$ (pressure $\times$ radius). Data in 30 experiments showed that each milliliter of oxygen consumption was associated with a tension of 4,000 dyne per centimeter per gram of heart weight. Coronary flow was dependent on oxygen uptake. An equivalence of the effects of pressure and of the cube root of the stroke volume in the calculation of the energy requirements of the heart was demonstrated. At constant pressures and outputs, doubling of the heart rate increased tension and oxygen uptake by about 60 per cent, in accord with the calculated increase in tension. A residual volume equal to the stroke volume increased tension by about 25 per cent, raising the oxygen requirements and reducing cardiac efficiency. Relative efficiency was related to the square of the radius of the heart.

In corollary studies on the spherical dynamics of the heart a linear relation was found between the cube root of the cycle length and the duration of

electrical systole: $QT = 0.5 \sqrt[3]{RR} - 0.125$. Other studies include an analysis of the role of tension in the induction of muscular hypertrophy, and re-examination of the "Law of the Heart." These contribute to the suggestion that certain aspects of the dynamics of the heart are determined by its geometry, rather than by changes in its metabolism.

97. THE EFFECT OF SODIUM PHYTATE ON THE FORMATION OF RENAL STONES IN PATIENTS WITH IDIOPATHIC HYPERCALCIURIA

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Seven patients with idiopathic hypercalciuria and a history of multiple renal stones for 6 years or longer were given 6 to 8 Gm. of sodium phytate orally in 4 divided doses per day for 1 year. Each patient was observed for at least 1 year before and 1 year after the administration of sodium phytate. All patients had normal carbon dioxide combining power; normal values for serum calcium, chloride, potassium, alkaline phosphatase, urea, and creatinine; low values for inorganic phosphate; and average urinary excretion of calcium ranging from 190 to 370 mg. per day while on an Aub diet. The renal stones consisted of calcium carbonate, calcium phosphate, or calcium oxalate.

While the patients were taking sodium phytate, the decrease in urinary calcium averaged 51 per cent per day (range: 37 to 74 per cent) and the increase in urinary phosphorus averaged 60 per cent per day (range: 45 to 69 per cent). Three patients had a slight increase in serum inorganic phosphate; otherwise, there was no change in the chemical constituents of the blood. Although the titratable acidity of the urine increased slightly and the ratio of urinary ammonia to urinary titratable acidity decreased, the changes were so small as to be of questionable significance.

Four patients had no renal stones when treatment with sodium phytate was started. Three remained free of stones during the year they took sodium phytate and during the subsequent year. In the other patient, 2 stones formed and passed during the year he took sodium phytate, and 2 more stones formed during the subsequent year.

Three patients had renal stones when treatment with sodium phytate was started. In all 3, the stones grew during the year sodium phytate was administered. During the same period one patient had 2 new stones, and another required surgical removal of a stone. In the third patient, the stone continued to grow after administration of sodium phytate was discontinued, and ultimately required surgical removal.

98. RESPONSE OF PULMONARY ARTERY PRESSURE OF PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA TO PROLONGED MILD EXERCISE

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In 16 men the existence of uncomplicated chronic obstructive emphysema was suspected by clinical findings and proved by the determined existence of

elevations of both total lung volume and functional residual capacity beyond the accepted upper limits of normal. Following cardiac catheterization and determination of the control central venous pressures and cardiac outputs (Fick), exercise was instituted at a steady, mild external work load of 850 foot pounds per minute employing a bicycle ergometer and continued for 39 minutes. Pulmonary artery pressure was recorded every minute except during the cardiac output determinations (periods of 9 to 14, 19 to 24, and 29 to 34 minutes). The data were grouped according to average resting mean pulmonary artery pressure (20 mm. Hg or less, 9 subjects; more than 20 mm. Hg, 7 subjects) and compared to a similarly studied group of normal untrained patients (11 subjects).

There was no significant difference among the 3 groups in the resting oxygen consumption, minute ventilation, arteriovenous oxygen difference, and cardiac index. Three emphysematous patients had normal resting mean pulmonary artery pressures (below 17 mm. Hg). There was a significantly greater increase in exercise pulmonary artery pressure among both emphysema groups compared to the normal, but no difference compared to each other. For the first 9 minutes of exercise none of the normal subjects achieved average mean pressure elevations above 18 mm. Hg, and none of the subjects with proved emphysema less than 19 mm. Hg.

The determination of a group of pressures during 9 minutes of a mild exercise offers better predictability and pulmonary-function correlation as to the existence of measureable emphysema, if uncomplicated by other cardiopulmonary disease, than the sole determination of resting pressures. In the limited number of patients investigated the exercise pressure–pulmonary function correlation was 100 per cent.

99. PENICILLIN "FALL-OUT" AS A FACTOR IN NOSOCOMIAL INFECTIONS

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(INTRODUCED BY DONALD W. SELDIN, M.D.)

Strains of *Staphylococcus aureus* permanently colonize the anterior nares of one-third to one-half of individuals. Permanent nasal carriers serve as a major focus from which staphylococci may be disseminated. Persons who are nasal carriers of staphylococci and are either hospitalized as patients or employed in hospitals acquire strains of staphylococci which are almost uniformly resistant to penicillin, whether or not they personally receive penicillin. This change in bacterial ecology has been attributed to a propensity for penicillin-resistant staphylococci to replace sensitive strains. However, natural replacement of penicillin-sensitive staphylococci by penicillin-resistant staphylococci has not been satisfactorily demonstrated. A selective environmental factor altering the normal host-parasite relationship in favor of penicillin-resistant strains of *Staph. aureus* would seem to be a more reasonable thesis.

Gould demonstrated particles of penicillin in air, dust, and fomites (Lancet 1: 489, 1958). The anterior nares of carriers of *Staph. aureus* were contaminated by inhalation of sufficient penicillin to inhibit penicillin-sensitive strains of *Staph. aureus*, thereby favoring colonization by penicillin-resistant strains.

Petri plates containing agar seeded with the strain of *Sarcina lutea* (A.T.C.C. No. 9341 employed for penicillin assays) were exposed to the air in various areas of the hospital. Simultaneous control plates into which penicillinase was incorporated were likewise exposed. Particles of penicillin ranging from minimal detectable quantities (less than 0.02 unit) to 0.8 unit fell upon the plates. The "fall-out" was greatest in nursing stations where syringes were filled with penicillin, in rooms in which patients were receiving penicillin, and in the pharmacy. Variation in fall-out for 4-hour periods over 24-hour periods of study corresponded only roughly to the times of administration. Penicillin was demonstrated in the anterior nares of the majority (63 per cent) of personnel present in areas where penicillin fall-out was demonstrated.

These observations support the hypothesis of Gould and suggest additional means by which nosocomial infections due to penicillin-resistant strains of *Staphylococcus aureus* might be controlled.

100. THE KETOGENIC EFFECT OF EXPOSURE TO COLD AND THE ANTIKETOGENIC EFFECT OF DEHYDRATION

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Ketosis associated with exposure to cold was first described by Sargent and Consolazio (1950) on the basis of a high incidence of positive Rothera reactions among healthy men in the winter. In the winter of 1954 and summer of 1955 two field investigations of survival rations provided quantitative data on 198 healthy men which have confirmed and extended this observation. The plan of these trials was identical. For 2 preperiod and 2 recovery weeks 99 subjects subsisted on a nutritionally adequate ration. In the experimental period (10 to 14 days) some of the men were fed ketogenic diets (starvation, high-fat, and low-carbohydrate) and some nonketogenic diets (pure carbohydrate and balanced regimen). Half of the men marched 12 miles per day; half only 3; half were allowed unlimited water; half only 910 ml. per day. For the last 2 days of the preperiod, the first 2 days of recovery, and daily during the experimental period aliquots of approximately 3,000 specimens of the 24-hour urinary excretion were analyzed quantitatively for total ketone bodies. Periodically when the subjects were resting and postabsorptive, timed specimens of blood and urine were obtained.

In summer, the rate of ketone body excretion (ketonuria) in resting and 24-hour specimens was equal; in winter, ketonuria was greater in resting urines. Both in summer and winter ketonuria was greater during cold rather than warm periods among subjects on nonketogenic diets. Even when the ketogenic-ketolytic ratio was constant, ketogenicity of ketogenic diets was greater in winter than summer, greater among men on unlimited water than limited, greater among men doing light work than hard, and greater at 1,000 calories per day than at 2,000. The onset of ketosis was delayed in summer, especially when water was limited. This delay and the antiketogenic effect of limited water were both correlated with degree of orthostatic hypotension. This body of evidence suggests that ketonuria might well serve as one measure of metabolic effects of cold on man and demonstrates that cold and dehydration are variables which must be taken into account in any study of ketone body metabolism.

101. A COMPARISON OF THE ANTIBODY RESPONSE IN SPLENECTOMIZED AND NONSPLENECTOMIZED HUMAN SUBJECTS

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Recent reports have suggested hypersusceptibility to infection in splenectomized infants and children. In this clinic splenectomized patients are predominantly adults or older children, and the incidence of serious infections, postsplenectomy, has not been remarkable. One of the factors which should be evaluated in this respect is the humoral antibody response. To date, relatively few intensive and conclusive studies have been done in this area.

A total of 105 splenectomized patients were immunized with the Foshay tularemia vaccine and 5 weekly antibody determinations performed. Age of the patients at time of immunization (2 months to 18 years postsplenectomy) varied from 2 to 77 years.

After 3 daily subcutaneous injections of 0.5 ml. of antigen, 52 of 57 splenectomized patients formed antibodies; titers and time of appearance were analogous to those in 32 controls who all formed antibodies. After a single injection of vaccine 42 of 48 splenectomized patients, as compared to 15 of 15 control subjects, formed antibodies. Except for a one-week lag period in some of the splenectomized group, titers were comparable subsequently to those observed in the control subjects. Thus, 12 of 105 splenectomized patients did not form antibodies. These included 1 of 48 with the thrombocytopenic purpura, 5 of 23 with congenital hemolytic anemia, 1 of 12 with acquired hemolytic anemia, 2 of 2 with hemolytic anemia secondary to chronic lymphatic leukemia, 1 of 8 with splenic neutropenia, 1 of 5 with splenic panhematopenia, 1 of 1 with aplastic anemia, and 0 of 6 splenectomized because of traumatic rupture of the spleen.

Electrophoretic analysis of serum proteins was performed on all specimens studied serologically. Of the 12 splenectomized patients not forming antibodies, 5 showed reduced gamma globulin, 6 increased, and 1 relatively normal globulin.

At present, these studies demonstrate that the absence of the spleen did not affect antibody formation in 88.6 per cent of 105 patients. Other factors such as the underlying disease process are more likely involved in the failure of antibody formation in 11.4 per cent of our group.

102. MEASUREMENT OF BLOOD FLOW IN MINUTE VOLUMES OF SPECIFIC TISSUES IN MAN

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Blood flow through a 4 to 5 c.mm. portion of tissue can be determined by measuring the rate of temperature change in the tissue before and after local arterial occlusion.

Before occlusion, the rate of change of heat content ($\dot{T}_t$) of tissue equals the rate heat is brought to it by the arterial blood and is produced by local metabolism, minus the rate at which heat is lost through venous blood drainage and net conduction to the surroundings. After occlusion, the factors of arterial and venous blood flow are eliminated, and a new rate of change ($\dot{T}_t'$)

exists. The difference between these two rates of change ($\dot{T}_t - \dot{T}_t'$) equals the rate heat was brought to and taken from the tissue by the blood. If the temperature (T_a) of the local arterial blood and that (T_v) of the local venous blood and the thermal capacities of the tissue (C_t) and blood (C_b) are known, then by the formula $F = \frac{C_t\ (\dot{T}_t - \dot{T}_t')}{C_b\ (T_a - T_v)}$ blood flow (F) may be calculated.

If it is assumed that T_v is the same as the local tissue temperature, all the factors for determining F are known except T_a. However, with an additional modified occlusion observation, the needed value T_a may be calculated.

The thermometer (sensitivity 4×10^{-4}° C. and response time 95 per cent equilibrium in 0.27 sec.) and heating unit are housed in a 25 gauge needle. Femoral arterial occlusion was performed manually.

Twenty-five observations of skin blood flow were made in 3 subjects with the combined thermometer and heating unit placed 1 to 2 mm. beneath the skin surface of the right calf. Flows ranged from 1.3×10^{-4} to 82×10^{-4} gram of blood per gram of skin tissue per second.

An extension of the technique has led to the measurement of the temperature coefficient of flow, when flow is influenced by very local temperature changes. In 9 observations a value of +39 per cent per degree centigrade was found.

Periodic fluctuations of the tissue temperature corresponding to the pulse and respiration have been seen.

103. PLASMA INSULIN ACTIVITY IN NORMAL AND DIABETIC MAN DURING HYPOGLYCEMIC RESPONSE TO ORINASE AND INDOLE-3-ACETIC ACID

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Orinase (tolbutamide) acts principally by directly stimulating endogenous insulin secretion. Conversely, Mirsky reports that indole-3-acetic acid (IAA), a physiologic end product of tryptophan metabolism, induces hypoglycemia by inhibiting insulinase activity. In normal subjects and in mild or severe diabetics, these substances with supposedly different metabolic mechanisms exerted identical effects on blood glucose and plasma insulin activity in the same patient.

Orinase (3 Gm.) or IAA (100 mg. per kilogram) was given orally to the following: 5 normal subjects; 7 elderly diabetics who responded excellently to Orinase therapy (Orinase-responders); 6 elderly diabetics who responded inadequately to Orinase (Orinase-nonresponders); and 5 severe juvenile diabetics. Plasma insulin activity was assayed by Vallance-Owen's rat diaphragm method.

Hypoglycemic Response.—Mean maximal depression of glucose (per cent fall from fasting value) was as follows. Normals: Orinase 22 ± 4 (s.e.m.), IAA 11 ± 4; Orinase-responders: Orinase 44 ± 4, IAA 35 ± 6; Orinase-nonresponders: Orinase 25 ± 5, IAA 24 ± 8; juveniles: Orinase 1 ± 1, IAA 7 ± 3.

Fasting Plasma Insulin Activity.—Normal subjects and all elderly diabetic patients showed significant fasting insulin activity (micrograms glucose uptake per 10 mg. diaphragm; mean ± s.e.m.): normals 178 ± 18 ($p < 0.001$); Orinase-responders 142 ± 14 ($p < 0.001$); Orinase-nonresponders 116 ± 18 ($p < 0.005$). Fasting insulin activity was absent in juveniles, 12 ± 12 ($p < 0.7$).

Plasma Insulin Activity After Orinase.—No group exhibited increased glucose uptake above fasting values: normals –4 ± 12 μg glucose uptake per 10 mg. diaphragm, above fasting uptake; Orinase-responders –2 ± 26; Orinase-nonresponders –48 ± 2; juveniles –54 ± 30.

Plasma Insulin Activity After IAA.—Similarly, no group showed significant variation from fasting glucose uptake: normals –34 ± 26; Orinase-responders 26 ± 22; Orinase-nonresponders 54 ± 52; juveniles –2 ± 30.

The data suggest that insulin-dependent hepatic mechanisms determine the hypoglycemic response to both Orinase and IAA. Binding of Orinase-released insulin within the liver probably precedes the reduced hepatic glucose output found by Recant and others. If liver-insulinase is inhibited by IAA, increased net intrahepatic insulin may be bound in situ and augment glucose assimilation while depressing its release. In each case, hypoglycemia can occur without enhanced insulin activity in peripheral blood.

104. EFFECTS OF INFUSION OF ACETYLCHOLINE ON PULMONARY VASCULAR RESISTANCE IN PATIENTS WITH PULMONARY HYPERTENSION AND CONGENITAL HEART DISEASE WHILE BREATHING AIR AND OXYGEN

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The aim of the present investigation was to determine the importance of vessel tone in the genesis of pulmonary hypertension associated with congenital heart disease. Acetylcholine, an effective vasodilator, was administered by constant-rate infusion into the pulmonary arteries of 10 patients who had pulmonary hypertension associated with atrial or ventricular septal defects. Rapid inactivation of acetylcholine in the circulating blood permits infusion of solutions of sufficient concentration to affect the pulmonary vessels without altering the hemodynamics of the systemic circulation. The amount used did not cause changes in cardiac rate or systemic pressure nor did it affect the pattern of respiration.

The oxygen consumption, oxygen saturation of pulmonary, arterial and radial arterial blood, and pulmonary arterial pressure were recorded continuously before, during, and after infusion. The magnitude of the venoarterial shunt, when present, was determined by dye dilution curves, so that the oxygen content of pulmonary venous blood could be estimated. These data then were used to determine changes in pulmonary vascular resistance during infusion of acetylcholine.

The pulmonary resistance was calculated in all patients while they breathed air and while they breathed 99.8 per cent oxygen. Eight of 10 patients showed a significant decrease in resistance during infusion of acetylcholine while breathing air and while breathing oxygen without acetylcholine. Administration of acetylcholine during inhalation of oxygen produced a further decrease in resistance.

It is concluded that these changes in pulmonary resistance represent vasodilatation of pulmonary vessels. The results indicate that activity of smooth muscle plays an important role in the maintenance of pulmonary vascular resistance in patients with pulmonary hypertension and congenital heart disease.

105. OPTIC NEURITIS ASSOCIATED WITH UNCONTROLLED DIABETES MELLITUS

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(INTRODUCED BY E. PERRY MCCULLAGH, M.D.)

After exclusion of all known causes of optic neuritis (optic atrophy) in diabetic patients, 14 patients with uncontrolled diabetes mellitus and optic neuritis were studied. Eleven of the 14 patients denied using tobacco. Only 2 of the 14 patients drank alcohol. The age of these patients varied from 40 to 69 years of age.

The chief complaint of all of these patients was recent loss of vision which led to the discovery of previously unknown moderate to severe diabetes mellitus in 12 of the 14 patients. The admitting blood sugar values were over 200 mg. per 100 ml. in all except one patient. In 7 of the 14 patients the blood sugar values were over 300 mg. per 100 ml. Thirteen of the 14 patients had routine urine examinations and showed 3 to 4 plus glycosuria. Symptoms of diabetes mellitus were recorded in 9 patients. Peripheral diabetic neuritis was present in 3 patients. Diabetic acidosis was not present in any of the patients.

The onset of the visual loss was gradual in 11 and rapid in 3 patients. The visual loss ranging from moderate to severe was bilateral in 8 and unilateral in 6 patients. Papillitis was observed in 8 and optic atrophy in 6 patients. Six patients showed central scotomas while 7 patients showed peripheral contraction of the visual fields. Control of the diabetes mellitus arrested the visual loss in all but one of the patients followed. In contrast, only one patient showed any significant improvement of vision after control of the diabetes mellitus.

On the basis of a few previous case reports of optic neuritis and uncontrolled diabetes mellitus and the clinical observations made in this much larger series of 14 patients, it is suggested that visual loss may be caused by the toxic effect on the optic nerves of uncontrolled diabetes mellitus in the occasional diabetic patient.

106. MEASUREMENTS OF ADRENOCORTICAL FUNCTION IN SUBJECTS RECEIVING ORAL 17-ACETOXYPROGESTERONE

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17-Acetoxyprogesterone (17-ACP), in amounts of from 20 to 40 mg. daily, is an effective oral progestational agent free of significant metabolic effects. In initial studies of its metabolism, 150 mg. were given daily to 4 subjects following suitable control observations. Pregnanediol and pregnanetriol remained either low or absent while 17-ketosteroid (17-KS) excretion increased slightly. However, 17-hydroxysteroid (17-OHS) excretion increased an average of 37 per cent over control values (modified Reddy procedure). In a fifth subject receiving 6-methyl-17-ACP, urinary 17-OHS excretion increased 75 per cent with no effect on 17-KS levels. One of several possible explanations is that 17-ACP can serve as a glucocorticoid precursor. Test of this thesis was pursued in view of general recognition that 17-hydroxyprogesterone is a cortisol precursor. Homeostatic mechanisms might obscure

evidence that 17-ACP is steroidogenic under influence of endogenous ACTH. Accordingly, additional experiments were performed in 6 normal volunteers given ACTH following oral loading with 17-ACP. Concentrations in plasma of free 17-OHS were determined at 2-hour intervals following 10 units of subcutaneous corticotropin gel. Both initial slopes and duration of increase were compared to those obtained when ACTH was given alone. 17-ACP appeared to augment ACTH in 4 subjects. In experiments on 5 additional subjects, both urinary and plasma steroid concentrations were determined in control periods and during both 17-ACP administration alone and with ACTH. Although urinary 17-OHS concentrations were again increased during administration of 17-ACP alone, neither plasma nor urinary 17-OHS response to ACTH was augmented. Average 17-KS excretion increased 4 mg. more with 17-ACP and ACTH than with ACTH alone.

The data for urinary and plasma 17-OHS responses to injected ACTH indicate that oral 17-ACP simultaneously given does not further enhance glucocorticoid formation. Further study is needed to explain the increased urinary 17-OHS concentration following 17-ACP ingestion alone and the greater increase in 17-KS excretion from ACTH when oral 17-ACP is given.

107. ATROPHY OF THE GASTRIC MUCOSA IN DOGS INDUCED BY A PREPARATION OF NORMAL HUMAN GASTRIC JUICE

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While confirming earlier work by Brunchseig and Code that intravenously administered preparations of normal human gastric content reduced volume and acid secretion in canine Heidenhain pouches, it was noted that some of the animals became permanently achlorhydric after repeated injections over a period of weeks. Autopsy studies on these animals revealed a pronounced atrophy of gastric mucosa, not only in the pouches, but also in the intact stomachs.

A controlled study of this phenomenon has now been carried out. Gastric mucosa was obtained from 7 healthy mongrel dogs by open biopsy. Twice weekly, six dogs were then given, intravenously, approximately 1 mg. per kilogram of body weight of dialyzed, lyophilized, pooled normal human gastric juice redissolved in normal saline. One dog was not injected. Repeated gastric biopsies were done at various intervals in all dogs.

After as few as 7 injections, atrophy of gastric mucosa was readily apparent. The changes consisted of thinning of the mucosa, disruption and disorganization of the glandular pattern, diminution in number of parietal cells, and increased connective tissue. With further injections, the changes became more pronounced until the glandular portion of the gastric mucosa had been virtually eliminated. The dogs were noted to be achlorhydric. No abnormality was noted in the biopsies from the control injected dog.

Blood counts are being followed in the animals. Anemia has not yet developed. Injections have been discontinued in 3 animals to ascertain whether the changes are reversible. It is clear that an experimental method of inducing atrophic gastritis in dogs has been devised. The biologic significance of the changes and the mechanism whereby they are brought about have not as yet been clarified.

108. OBSERVATIONS ON THE TREATMENT OF HEPATIC COMA

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Hepatic coma resulting from any one of several factors or a combination of them is, at present, still a very ominous state and for which, as yet, only little has been accomplished therapeutically. Hence, any new therapeutic agent that offers some possible hope in alleviating this state is readily accepted and tried.

In this report, we wish to present our clinical experience with 30 cases of hepatic coma—mostly in patients having Laennec's cirrhosis—who were given large doses of steroids plus monosodium L-glutamate (100 ml. being equivalent to 25 Gm. L-glutamic acid) or a mixture of 12.5 Gm. L-arginine and 11.25 Gm. of L-glutamic acid, as mono-L-arginine-L-glutamate. These substances were given in addition to the usual therapeutic regimen consisting of balanced electrolytic fluids, vitamins, broad-spectrum antibiotics, laxatives, enemas, low-protein foods (in precoma states) and removal of any ammoniagenic substances.

Of the 30 patients, 24 died, 3 recovered sufficiently to be discharged from the hospital, and 3 recovered but are still in the hospital after 4½, 3½, and 3 months, respectively. Among the 24 patients who died, 16 originally showed improvement, but then lapsed into coma and died.

The implications obtained from this study will be discussed in relation to future therapeutic procedures.

109. A DEFECT IN THE PHAGOCYTOSIS ACCELERATING SYSTEM OF SOME HUMAN PLASMAS AS REVEALED BY THE BACTERICIDAL TEST FOR TYPE-SPECIFIC ANTIBODY TO GROUP A STREPTOCOCCI

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Group A streptococci are rapidly phagocytized and destroyed when they are grown in fresh human blood that contains antibody to M protein of homologous serologic type. This observation is the basis of the bactericidal test for type-specific streptococcal antibody. Complement and other accessory normal plasma factors, as yet not clearly defined, are necessary to promote the rapid phagocytosis which is essential to demonstrate the bactericidal effect in this system.

Two apparently healthy human beings were encountered, among 30 studied, whose bloods behaved atypically in that they failed consistently to produce strong bactericidal tests in the presence of anti-M antibody. The abnormality in these bloods was associated with the plasma rather than the blood phagocytes. Washed leukocytes from the two atypical bloods showed strong bactericidal activity when suspended in normal human plasmas. Conversely, washed normal human leukocytes showed poor bactericidal activity when suspended in the plasmas of these atypical bloods.

Time curves of the rate of phagocytosis during the 3-hour period of the test revealed a characteristic abnormality of the atypical bloods. Whereas normal human bloods showed rapid onset of phagocytic activity within 30 to 60 minutes, the atypical bloods showed a marked phagocytic lag during the first 1 to 2 hours. This lag could account for rapid extracellular proliferation of streptococci and, thus, an apparently poor bactericidal test.

Both atypical plasmas contained normal levels of whole complement and of all 4 complement fractions. Properdin was found to be an essential component of the phagocytosis accelerating system of plasma in the bactericidal test. The atypical plasmas, however, showed normal properdin activity by various methods. Their abnormal behavior did not appear to be related, therefore, to the properdin complement system.

The atypical bloods described apparently reflect defects in components of the phagocytosis accelerating system of human plasma that have not been defined previously. The bloods of both individuals have not changed in their behavior during repeated studies over periods of 1 and 2 years, respectively.

110. 6-AMINONICOTINAMIDE IN DISSEMINATED HUMAN CANCER

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The effect of administration of 6-aminonicotinamide (6-AN), a synthetic antagonist of niacin, has been studied in 26 patients, 19 of whom had carcinoma of the breast.

Reactions to this drug in the order of frequency have been: anorexia, nausea and vomiting, malaise, cheilosis and horizontal fissures, stomatitis, eighth nerve deafness, headaches and mental depression, conjunctivitis and uveitis, and depressed thyroid function. All these effects, with the exception of the eighth nerve injury, were reversible on withdrawal of the drug. The effect on thyroid function was noted only after the appearance of clinical myxedema in several patients. Euthyroidism could be produced by replacement therapy even though 6-AN was continued.

Tumor tissue obtained by biopsy from patients before and during 6-AN therapy revealed that the in vitro incorporation of C^{14} formate into tissue slices was markedly inhibited. The addition of 6-AN to the incubation media also caused a depression of C^{14} uptake, except in tissues from patients showing tumor regression during 6-AN therapy.

Since most of these studies were done in patients with far-advanced disease, evaluation of tumor regression could not be measured clinically. However, clinical evidence of tumor regression has been documented in a few patients with less-advanced disseminated cancer of the breast demonstrated by decrease in size of the soft tissue lesions and recalcification of osteolytic lesions.

111. THE URICOSURIC EFFECT OF CERTAIN ORAL ANTICOAGULANT DRUGS

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(Introduced by Muriel C. Meyers, M.D.)

The anticoagulant drugs, ethyl biscoumacetate (Tromexan) and bishydroxycoumarin (Dicumarol) have recently been shown to have uricosuric properties. Salicylates have long been known to have both anticoagulant and uricosuric effects.

The uricosuric action of single oral doses of 2 short-acting coumarin preparations (2,000 mg. ethyl biscoumacetate; 20 mg. acenocoumarin) and of 2 short-acting indandione preparations (300 mg. anisindione; 20 mg. phenylindandione) was measured in 4 healthy male subjects on a constant diet and

was compared to the effect of a single oral dose of probenecid, 1,500 mg. Urine and serum uric acid levels were determined both by the method of Block and Geib and by the uricase method; the results with these two methods agreed within 0.1 mg. per cent in each instance.

Each drug, in the dose given, had a similar effect on the aspects of blood coagulation measured (prothrombin, one- and two-stage methods, Factor VII, Factor V, clotting time, and thromboplastin generation time) and uniformly resulted in reduction of the Quick one-stage prothrombin concentration to between 40 and 50 per cent. A rise in urate clearance of over 50 per cent with a fall of serum uric acid of over 25 per cent was considered evidence of a uricosuric effect. By these criteria ethyl biscoumacetate (342 and 50 per cent), probenecid (234 and 47 per cent), and phenylindandione (88 and 30 per cent) were found to have a uricosuric action, while anisindione (22 and 15 per cent) and acenocoumarin (34 and 3 per cent) lacked this effect in the doses employed.

112. THE EFFECT OF HIGH PERFUSION PRESSURES ON THE GRANULATION OF JUXTAGLOMERULAR CELLS IN AN ISOLATED KIDNEY

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Twenty-one isolated normal kidneys were perfused with oxygenated "biochemically supported" blood at pressures ranging between 167 and 192 mm. Hg. The degree of granulation of the renal juxtaglomerular cells (the juxtaglomerular index) dropped on the average from 44 to 26 during the 1- to 4-hour perfusion at these high pressures. Sixteen similar isolated normal kidneys were perfused at pressures ranging between 82 and 85 mm. Hg. In these kidneys, the average juxtaglomerular index was 43 at the start of the perfusion and still remained 43 at the end of the perfusion. The difference between the two groups was highly significant ($p < 10^{-12}$). Thus, high perfusion pressures produce marked degranulation of the juxtaglomerular cells in a short period of time, while perfusion at fairly normal pressures produces no change in juxtaglomerular granulation.

Supplementary experiments indicate that the degranulation of juxtaglomerular cells at high perfusion pressures is not caused by an augmented secretion of the adrenal, is not caused by the release of humoral substances into the renal vein, and is not related to the degree of hemolysis in the experiment.

These observations are compatible with the theory that the juxtaglomerular cells can act as baroreceptors, changing their rate of secretion as the level of arterial pressure changes.

113. PANCREATIC EXOCRINE FUNCTION IN DIABETES MELLITUS AND CIRRHOSIS OF THE LIVER

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(Introduced by Goronwy O. Broun, Sr., M.D.)

Because of a high incidence of structural abnormalities found in the exocrine pancreas in patients with cirrhosis of the liver and diabetes mellitus,

this study was undertaken to determine the incidence of abnormalities of pancreatic exocrine function in patients with these diseases. Nineteen patients with diabetes mellitus (who had nothing clinically to suggest pancreatic acinar disease) and 13 patients with cirrhosis of the liver were studied as of this date by means of a "pancreatic profile"; these results are compared to those found in 22 patients known to have carcinoma of the pancreas or chronic pancreatitis.

This pancreatic profile consisted of at least 3 determinations of serum diastase, 3 determinations of 24-hour urinary diastase excretion, serum diastase response to secretin, and duodenal drainage studies after secretin. Duodenal contents were analyzed for volume, maximum concentration of bicarbonate, total amount of bicarbonate, amount of diastase, and concentration of trypsin. Normal values were calculated for each of these as the mean ± 2 S.D.

The percentage of diabetic patients with abnormalities were as follows: serum diastase, 53; urine diastase, 56; serum response, 42; duodenal volume, 16; maximum bicarbonate concentration, 5; total bicarbonate, 16; amount of diastase, 5; trypsin, 31.

The percentage of abnormalities in the cirrhotics were: serum diastase, 73; urine diastase, 50; serum response, 55; duodenal volume, 36; maximum bicarbonate concentration, 0; total bicarbonate, 18; amount of diastase, 0; trypsin, 27.

In 22 patients with proved chronic pancreatitis or carcinoma of the pancreas the percentage of abnormalities were: serum diastase, 68; urine diastase, 59; serum response, 50; duodenal volume, 36; maximum bicarbonate concentration, 55; total bicarbonate, 64; amount of diastase, 14; trypsin, 14.

While the exact significance of the data may be controversial, it is suggested from these studies that a high incidence of abnormalities of pancreatic acinar function occurs in diabetes mellitus and cirrhosis.

114. EFFECTS OF EXCHANGE OF BLOOD BETWEEN NEPHROTIC AND NORMAL RATS: EVIDENCE AGAINST DYSPROTEINEMIA AS A CAUSE OF PROTEINURIA

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The concept that there is a change in plasma proteins (dysproteinemia) in proteinuria is the basis for one of the major explanations for loss of proteins into the urine. In a recent study we have attempted to assess the validity of this concept by producing experimental nephrosis in rats and then exchanging the blood between normal and nephrotic rats at the peak of nephrosis in the latter. Also, the proteins from the urine of the nephrotic rats were extracted and were given in Ringer's solution intravenously to another group of normal rats. In the normal and the nephrotic rats, the pattern and quantity of urinary and plasma proteins were determined electrophoretically in the control period and at frequent intervals before and after performance of the exchange transfusion of blood or the intravenous administration of the extracted urinary proteins. Nephrosis was produced in the rats by injection into the tail vein of serum from rabbits sensitized to rat kidney. The urine was collected continuously, subsequent to cystotomy, by use of a polyethylene tube with mushroom tip as an indwelling catheter in the bladder. The rats were kept in individual cages and could be maintained in excellent condition throughout the study period of at least 1 week after the exchange transfusion.

In spite of the fact that the exchange transfusion was made at the peak of proteinuria and hypoproteinemia, the nephrotic rats receiving the normal blood from their litter mates continued to lose large amounts of plasma proteins into the urine, while the normal rats receiving nephrotic blood from their nephrotic litter mates did not show any evidence of increased loss of plasma protein into the urine. Furthermore, the normal rats that received intravenously in Ringer's solution the proteins extracted from the urine of their nephrotic litter mates did not show any increase in proteinuria. The repeated findings that neither blood from nephrotic rats nor protein extracted from the urine of nephrotic rats caused any additional loss of protein into the urine of normal rats are considered convincing evidence against the hypothesis that a change in plasma proteins is the cause of proteinuria.

115. DEMONSTRATION OF HEPATIC MAXIMUM REMOVAL CAPACITY (L_m) FOR GALACTOSE IN MAN

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If the liver has a maximum capacity to remove a substance from the blood which cannot be exceeded regardless of load, but which results in 100 per cent removal when the load is below maximum, it should be possible to estimate both the maximum removal capacity (L_m) and the hepatic blood flow (HBF) from analysis of the peripheral disappearance curve of the substance after single intravenous injection, without resort to hepatic vein catheterization (Am. J. Digestive Dis. 3: 137, 1958). The present study demonstrates that the liver has such a maximum removal capacity for galactose.

In each of 12 subjects without liver disease, two consecutive intravenous constant infusions of galactose, at different rates were delivered by a motor-driven syringe until peripheral concentrations (P) during each infusion attained equilibrium (P_{eq}). The removal rate of galactose (R) could then be calculated as: known infusion rate (I) minus urine excretion rate (U) (the equilibrium minimizing error due to mixing, diffusion, and size of volume of distribution). It was found that R is proportionate to I until $R \cong 500$ mg. per min. (when $P_{eq} \cong 50$ mg. per cent). Thereafter, increased I results only in higher values for P_{eq} and U, *without effecting further increase in R.*

Constant renal clearance for galactose was demonstrated by proportionate U and P_{eq} values at almost all levels. By hepatic vein catheterization in other subjects it was shown that when P is less than 50 mg. per cent, hepatic extraction of galactose is complete; when P is greater than 50 mg. per cent extraction is incomplete.

A small amount of extrahepatic, extraurinary removal of galactose can be deduced from consideration of P_{eq} versus calculated hepatic blood flow (calculated by the method of Tygstrup and Winkler, 1958) for each pair of infusions in each individual. Nevertheless, since R is due in such large part to hepatic removal, the demonstration of a maximum for R demonstrates a hepatic removal maximum (L_m) for galactose. Therefore, galactose appears to fit the major requirements for a test substance for the single injection, peripheral disappearance method for estimating L_m and HBF.

116. THE VECTORCARDIOGRAPHIC QRSsÊ LOOP FINDINGS IN CHRONIC COR PULMONALE

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Vectorcardiograms were recorded with Grishman's cube lead system from 42 patients with pulmonary emphysema. Four distinctive QRSsÊ loop patterns were observed in the study series, the salient features of which were as follows:

Type A Pattern.—A prominent terminal deflection of the loop to the right, posteriorly and superiorly, never exceeding the earlier leftward extent of the loop.

Type B Pattern.—A large terminal deflection of the loop, the posterior extent of which exceeded the slightly rightward, earlier leftward, and superior or inferior components of the loop.

Type C Pattern.—A large rightward, posterior, and usually superior terminal deflection of the loop, clockwise inscribed in the horizontal projection, whose rightward extent exceeded the earlier leftward extent of the loop.

TABLE I

QRSsÊ LOOP PATTERNS	TYPE A	TYPE B	TYPE C	RIGHT VENTRICULAR HYPERTROPHY
No. in group	10	16	7	9
Clinical chronic cor pulmonale	3	14	7	8
Cardiac X-Ray Findings				
Normal	8	5	4	2
RVE	2 (RVE & LVE)	0	1	3
Chronic cor pulmonale	0	11	2	4
ECG Findings				
Normal	4	5	0	0
sÂQRS: posterior, inferior, and medial	6	4	1	1
sÂQRS: right, posterior, and superior	0	7	3	1
RVE	0	0	3	7
Autopsied Cases (all with anatomic RVH)	0	3	2	3

RVH Pattern.—The same as described by Grishman and associates and by other observers. In Table I the QRSsÊ loop patterns are related to other clinical findings in the patients in the study series. Two autopsied cases in the Type C group with anatomic right ventricular hypertrophy had normal hearts on roentgenographic study as did one of the autopsied patients in the Type B group.

The authors believe that the Types B and C QRSsÊ loop patterns in chronic cor pulmonale reflect pulmonary outflow tract hypertrophy. The transition of these loop patterns into loops typical of RVH has been observed in serial vectorcardiograms and less frequently in single vectorcardiograms during the respiratory cycle. The significance of these observations with reference to the genesis of the Types B and C QRSsÊ loop patterns will be demonstrated.

117. THE VECTORCARDIOGRAPHIC QRSsÊ LOOP FINDINGS IN INFEROPOSTERIOR MYOCARDIAL INFARCTION

THOMAS J. WALSH, M.D., AND GONZALO T. ROMAN, JR., M.D., ST. LOUIS, MO.

(INTRODUCED BY JOHN R. SMITH, M.D.)

Vectorcardiograms were obtained with Grishman's cube system of electrode placement on 50 patients with clinically established or autopsy proved inferoposterior myocardial infarction. The study series was made up of 25 cases of diaphragmatic infarction, 18 of posterolateral infarction, 9 of diaphragmatic-posterolateral infarction, and 8 cases of strictly posterior infarction.

The normal QRSsÊ loop is characterized by the following features. (1) Maximal mean instantaneous vector—left, inferior, and slightly posterior. (2) Mean 0.02 sec. instantaneous vector—to the left of +100 degrees in the horizontal plane, and inferior to –40 degrees in the right sagittal and frontal planes. (3) Direction of inscription—counterclockwise in the horizontal plane, clockwise in the right sagittal plane, and variable in the frontal plane.

Strictly posterior infarction, unlike the other types, could be recognized vectorcardiographically only by changes in the configuration and direction of inscription of the QRSsÊ loop. The characteristics of the loop were as follows. (1) Anterior displacement of the long axis and afferent limb of the QRSsÊ loop. (2) Total or partial reversal in the direction of inscription of the horizontal and/or right sagittal loops. (3) A terminal deflection of the QRSsÊ loop to the right and anteriorly or posteriorly.

A brief résumé of the vectorcardiographic findings diagnostic of the other types of inferoposterior infarction is presented in Table I.

TABLE I

QRSsÊ LOOP FNDINGS	DIAPHRAGMATIC INFARCTION	POSTEROLATERAL INFARCTION	DIAPHRAGMATIC-POSTERO-LATERAL INFARCTION
Displacement of efferent limb of loop	Superiorly	Rightward and anteriorly	Rightward, superiorly and anteriorly
Orientation of mean 0.02 sec. vector	Right sagittal and frontal: superior to –40°	Horizontal and frontal: to the right of +90°	Horizontal: +100° to +150° Right sagittal: –90° to –40° Frontal: –170° to –95°
Direction of inscription	Right sagittal: counterclockwise Frontal: clockwise	Usually not present	Frontal: clockwise

The mechanisms which have been postulated for the abnormalities of the QRSsÊ loop of the vectorcardiogram in inferoposterior infarction will be considered. In addition, certain advantages offered by the vectorcardiogram in establishing the diagnosis of inferoposterior infarction will be presented.

118. PERMANENT GRANULOCYTOSIS IN MICE DUE TO CORTISONE

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In the absence of bacterial infection or necrosis, temporary granulocytosis with or without lymphopenia occurs in mammals under a variety of circumstances, including treatment with hormones, vitamin deficiency states, stress,

and injection of nonspecific substances. Permanent and irreversible granulocytosis has heretofore been produced by only two agents—benzene and x-irradiation. The incidence of myeloid leukemia is increased by chronic benzene poisoning and by long-term or very heavy exposure to x-irradiation.

In mice, short-term cortisone treatment produces marked granulocytosis and myeloid metaplasia as well as lymphopenia. This led us to test the effect of intermittent long-term cortisone therapy on mice, hoping that permanent and irreversible changes might occur. White mice of a nonleukemic strain were given daily injections of 0.5 mg. of cortisone for 134 days in 14 periods of 6 to 15 days' duration, averaging 9.6 days, with rest periods between treatments averaging 21.5 days. The total time of intermittent treatment was 59 weeks. Control animals were maintained on the same injection schedule but were given the suspending vehicle for the cortisone.

During each treatment period in the early stages of the experiment the expected granulocytosis and lymphopenia occurred when cortisone was given with reversion to normal on discontinuing the drug. By the fifty-ninth week, when treatment was discontinued, the granulocytosis had become permanent in the experimental animals and persisted for the lifetime of the survivors. As compared to the controls, the granulocytosis was particularly marked between the seventy-third and ninety-third weeks. After the treatment period the lymphocyte counts returned to normal.

Two mice which survived 55 and 57 weeks under a similar experimental regimen showed histologic findings which were entirely comparable to human myeloid leukemia. This did not occur in the control animals and has not previously been found in our colony.

The mode of action of cortisone in producing a permanent change in the basic mechanisms which control the production of granulocytes or their release from the bone marrow is unknown. The metabolic change may be related to inhibition of vitamin B_6 activity.

119. RESINS FOR PREVENTION OF HYPERKALEMIA: AN ADVANCE IN THE TREATMENT OF ACUTE RENAL FAILURE

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Hyperkalemia occurring during the course of acute renal failure has been a major indication in the past for performing hemodialysis. The efficacy of the sodium cycle carboxylic acid type resin in lowering the serum potassium level has to a large degree obviated the necessity for removing potassium by lavage or dialysis. During the past two years, 14 patients with acute renal insufficiency due to various causes have been given this resin to control a rising serum potassium level. In only 3 of these patients did hyperkalemia become a significant feature of their disease. Reasons for failure of the resin to control the serum potassium level appeared to be: insufficient time for its action, inadequate dosage in the face of massive release of intracellular potassium, and inability of the patient to take or retain sufficient amounts of the resin. In the treatment of this condition an adequate dosage must be given early. Other effects of the resin have been: a partial correction of the metabolic acidosis occurring in the majority of cases, a moderate increase in the serum sodium level, a tendency to develop hypokalemia following diuresis or prolongation of resin therapy, and, very rarely, difficulty with rectal impaction.

The alleviation of the metabolic acidosis by the sodium cycle resin is in marked contrast to the accentuation of acidosis by ammonium or hydrogen cycle resins. Studies on normal fasting volunteers show that a single oral dose of 20 Gm. of the sodium cycle carboxylic acid resin lowers the plasma potassium concentration about 0.5 mEq. per liter in 4 hours. Some decrease in plasma calcium also occurs. There is a considerable elevation of the plasma pH at 4 hours. The greater effectiveness of oral resin, as compared to its rectal administration, may be in part the result of the intracellular shift of potassium secondary to the alkalosis resulting from the removal of hydrogen ions from the gastric juice.

120. MYOCARDIAL PROPERTIES IN EXPERIMENTAL CARDIAC ENLARGEMENT AND HYPERTROPHY

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Information concerning effects of cardiac enlargement on fundamental properties of myocardium is not available. In the course of studies of such properties under a variety of experimental conditions we have collected data allowing comparison of in vitro electrical and mechanical properties of left ventricular columnae carneae of the albino rat following cardiac enlargement induced by (1) exercise, (2) renal hypertension, and (3) hyperthyroidism. Growing rats exercised for 8 weeks showed an increase in heart-body weight ratio of 16 per cent. True histologic hypertrophy was not apparent. Excitability to square wave stimuli was increased in exercised myocardium, and maximum tension production per milligram of muscle was greater than normal by about 20 per cent. Rats with renal hypertension of 6 months' duration and those made hyperthyroid by thyroxine injection or thyroid feeding for 3 weeks showed a cardiac enlargement of approximately 30 per cent. Significant enlargement of fiber diameter was present. Refractory periods, excitability, and passive length-tension relationships were not abnormal in these groups. Maximum active tension production per milligram of muscle was not significantly modified in hypertensive hypertrophy but hyperthyroid myocardium showed a decrease of 25 per cent from normal. Nitrogen and water content were unchanged in all groups. These results indicate significant differences in functional significance of cardiac enlargements of varying etiology and suggest a spectrum of changes extending from the "work" hypertrophy of exercise through the metabolically defective hypertrophy of hyperthyroidism.

121. CHLORPROPAMIDE IN THE MANAGEMENT OF DIABETES MELLITUS

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(Introduced by Raymond W. Monto, M.D.)

Chlorpropamide (*N*-propyl-*N'*-p-chlorobenzenesulfonylurea) has a significant hypoglycemic effect in man. Recent experience with its use in the management of diabetes mellitus is the basis of this report.

Chlorpropamide has been used in 16 patients with diabetes mellitus. All of these patients have been followed from 1 to 6 months. This group includes 10 patients previously regulated on insulin in a daily dosage range of 12 to 70

units. Six other patients required 1.0 to 3.0 Gm. of tolbutamide daily. Fourteen of these 16 patients have been successfully controlled with chlorpropamide alone. Two patients previously well controlled on insulin were unsuccessfully regulated with chlorpropamide alone. Both patients required reinstitution of insulin because of progressive ketosis.

Chlorpropamide is initiated in dosages of 250 to 500 mg. daily, while the final maintenance dosage is dependent upon subsequent blood sugar levels. Nine of the 14 patients are currently maintained on 250 mg. daily; 3 patients require 500 mg., and 2 patients require 750 and 1,000 mg. daily, respectively, for good control. Good control of diabetes includes aglycosuria, fasting normoglycemia, and postprandial blood sugars under 150 mg. per cent. A subjective sense of well-being must also be maintained.

Toxicity has been minimal. One patient noted nausea and epigastric burning while on 500 mg. per day. A decrease in daily dosage to 250 mg. alleviated these symptoms. Another patient developed significantly positive liver flocculation tests without clinical manifestations of liver disease. Chlorpropamide was stopped in this patient and progress liver tests were normal. Careful renal, hepatic, and hematologic examinations in the remaining patients have disclosed no abnormality. No dermatologic complications have occurred.

From our brief experience, chlorpropamide appears to be a hypoglycemic agent that justifies further clinical investigation. Like tolbutamide, its application probably will be limited to the adult-type diabetic. Our two clinical failures have been in the juvenile-type diabetic patient. Further clinical experience will establish this point.

122. THE EFFECT OF TOLBUTAMIDE ON GLUCOSE AND NITROGEN METABOLISM IN THE TOTALLY DEPANCREATIZED DOG

HENRY L. WILDBERGER, M.D. (BY INVITATION), AND HENRY T. RICKETTS, M.D., CHICAGO, ILL.

Contrary to the view that the presence of pancreatic beta cells is necessary to the action of tolbutamide is the finding previously reported by us (Ann. New York Acad. Sc. 71:170, 1957) of a reduction by tolbutamide of blood and urinary glucose in totally depancreatized dogs. These animals received small suboptimal amounts of insulin necessary to maintain life. The present experiments were undertaken to determine whether the lowering of blood and urinary glucose is attributable to potentiation of injected insulin.

Mongrel dogs were completely depancreatized, kept in metabolism cages, and maintained throughout the following procedures on a constant diet and two small doses of crystalline insulin daily. Observations were carried out during alternating control and experimental periods, each of 2 weeks' duration. Addition of 0.25 Gm. of tolbutamide twice daily by mouth resulted in a significant decrease of blood sugar and urinary glucose as described previously, but nitrogen excretion was not affected. Addition of 2 to 4 units of protamine zinc insulin instead of tolbutamide produced a diminution of blood and urinary glucose comparable to that given by tolbutamide and a distinct fall in nitrogen excretion.

Since under these conditions tolbutamide does not cause nitrogen retention whereas extra insulin does, it is concluded that the oral drug does not

act by potentiating the maintenance doses of injected insulin. It seems likely that the primary action of tolbutamide takes place in the liver, the functional integrity of which requires a certain minimum of insulin.

123. A STUDY OF THE NUCLEIC ACID COMPOSITION OF THE BONE MARROW OF PATIENTS WITH PERNICIOUS ANEMIA

JOHN J. WILL, M.D. (BY INVITATION), HELEN S. GLAZER, PH.D. (BY INVITATION), AND RICHARD W. VILTER, M.D., CINCINNATI, OHIO

Studies of the nucleic acid composition of bone marrow cells of patients with megaloblastic anemias have indicated that the concentrations of ribose nucleic acid (RNA) and uracil are high compared with desoxyribose nucleic acid (DNA) and thymine. After treatment with vitamin B_{12} or folic acid, normal relationships are restored. To substantiate these relative changes in nucleic acid composition of bone marrow of patients with megaloblastic anemias, we have determined the absolute amounts of the nucleic acids and their purine and pyrimidine bases per nucleated bone marrow cell.

Twenty-one patients with pernicious anemia have been studied. Total nucleated cell counts were made on buffy coat suspensions of bone marrow aspirates. RNA and DNA were measured in these buffy coat suspensions by the orcinol and diphenylamine reactions. Uracil, thymine, cytosine, adenine, and guanine were determined by a paper chromatographic procedure adapted to bone marrow analysis. In 7 patients, these determinations were repeated on marrow specimens obtained after treatment with vitamin B_{12}. The bone marrow from 11 normal subjects served as controls.

The following results have been obtained. (1) In pernicious anemia in relapse the cellular content of RNA is increased, while that of DNA is altered insignificantly. The average bone marrow cell contains 0.5×10^{-8} mg. of RNA and 0.6×10^{-8} mg. of DNA as compared with 0.2×10^{-8} mg. and 0.6×10^{-8} mg., respectively, in the average bone marrow cell of normal individuals. (2) The purine and pyrimidine bases are increased also. Thymine is altered the least. Average values for thymine, uracil, cytosine, adenine, and guanine expressed in micromoles per bone marrow cell are 0.6×10^{-8}, 0.15×10^{-8}, 0.6×10^{-8}, 0.8×10^{-8}, and 0.7×10^{-8}, respectively, as compared with 0.4×10^{-8}, 0.06×10^{-8}, 0.4×10^{-8}, 0.5×10^{-8}, and 0.4×10^{-8}, respectively, in the average bone marrow cell of normal subjects. (3) The magnitude of the biochemical alterations correlates with the degree of megaloblastosis of the bone marrow cells. (4) Vitamin B_{12} therapy decreases the cellular content of total nucleic acids and their purine and pyrimidine constituents primarily because of a decrease in RNA and uracil content.

These results provide qualitative and quantitative data of the effect of vitamin B_{12} upon nucleic acid composition in human beings.

124. APLASTIC ANEMIA

A REVIEW OF THIRTY-THREE PATIENTS

SLOAN J. WILSON, M.D., WILLIAM E. LARSEN, M.D. (BY INVITATION), AND JOHN S. TROMBOLD, M.D. (BY INVITATION), KANSAS CITY, KAN.

Aplastic anemia has been observed in 33 patients during the past 12 years. Only those patients were included in the study in whom there was

no diagnostic confusion with other blood dyscrasias. The causes of the severe marrow depression were most difficult to establish, being possibly determined in 18 subjects and included benzine, sulfonamides, chloramphenicol, aniline dyes, and the halogenated hydrocarbons. The sex incidence was 15 females and 18 males. The age incidence ranged from 5 to 79 years.

Therapeutic measures could be divided into 3 types: (1) massive folic acid therapy in the period of 1946 to 1950; (2) ACTH and corticosteroids, vitamin B_{12}, and folic acid from 1950 to 1958; and (3) splenectomy. Only one of 8 patients recovered using massive folic acid therapy. When ACTH and corticosteroids were used, 5 of 25 patients recovered, one of whom also had a splenectomy. Splenectomy was done in 3 other selected cases in addition to using ACTH, corticosteroids, vitamin B_{12}, and folic acid, but none recovered. Bone marrow observations with an interpretation of early attempts of the formed elements to regenerate gave a false optimistic impression as to the ultimate fatal outcome. In those patients who recovered, the bone marrow would at times have an extreme degree of hypoplasia even though the peripheral blood-formed elements would be adequate. The platelets were the last-formed element to regenerate. The most frequent cause of death was hemorrhage.

It would seem that the best results have been obtained with ACTH and corticosteroids in combination with other supportive measures. The indications and contraindications for splenectomy are not entirely clear. Aplastic anemia is still a dire disease, and modern therapy is still woefully inadequate.

125. INTRACELLULAR COMPOSITION AND RENAL RESPONSE DURING INDUCED EXTRACELLULAR OSMOL DEPLETION

SAMUEL ZALTSMAN, M.D. (BY INVITATION), ELIZABETH LOPEZ, M.D. (BY INVITATION), AND JACK METCOFF, M.D., CHICAGO, ILL.

The apparent renal failure and lack of cellular response accompanying acute severe hypotonicity were studied in 10 dogs depleted of sodium and water by in vivo dialysis. Analyses of muscle biopsies were compared with measurements of discrete renal functions during the 6- to 8-hour study periods while body water was slightly depleted and extracellular osmolality was reduced from 300-310 to 260-270 mOsm. per liter.

Acute hypotonicity was characterized by marked reduction of renal blood flow (C_{PAH}) and glomerular filtration (C_{IN}). The filtration fraction was not changed significantly. Relative renal ischemia could not be prevented by elevation of systemic blood pressure or volume. C_{H_2O} often represented more than 10 per cent of glomerular filtrate at the small urine volumes and C_{OSM} observed. Virtually complete reabsorptions of sodium and chloride were noted.

Muscle cell composition seemed unaffected by marked extracellular hypotonicity. This could not be attributed to the renal response. Assuming an extracellular position for chloride, either osmotic inequality was tolerated by the cell or dissociation of some intracellular constituent was changed, since contents (mM. per 100 Gm. DFFS) of K, Mg, and P were unaltered. Alternatively, assuming isosmolality of cell fluid accomplished by intracellular accumulation of water, subsequent calculations indicated that the intracellular concentrations of Na and Cl increased, while those of K, Mg, and P decreased.

Interpretations of the renal response are compatible with a modified hypothesis of counter-current flow in the medullary system and contingently

decreased concentrations of energy-linked intracellular P and Mg with secondary alteration of membrane permeability to Cl and Na—perhaps in the final concentrating segment of the tubule. Reduced solute filtration, slow transit time, diminished medullary blood flow, and trapping of Na and Cl in the medullary interstices would enhance reabsorption of water from the collecting ducts and account for the small volume of dilute urine or virtual anuria which occasionally represents maximal renal defense in acute iatrogenic hypotonicity. Extrarenal defense of cell integrity must be temporarily achieved by intracellular accumulation of water, reduced concentrations of energy-related ion substrates, intracellular accumulation of Na, and increased membrane permeability for chloride.

126. THE NATURE OF BLOCKING ANTIBODIES IN HUMAN BRUCELLOSIS

Horace H. Zinneman, M.D. (By Invitation), Harry Glenchur, M.D. (By Invitation), and Wendell H. Hall, M.D., Ph.D., Minneapolis, Minn.

The presence of a prozone phenomenon of no agglutination in low serum dilutions has been noted in the agglutination test for brucellosis. It is absent in the serum of patients with acute infections and appears in the subacute and chronic stage of the disease. Addition of prozone sera to agglutinating sera blocks or inhibits agglutination of brucella. In some cases this blocking titer may become high enough to blot out all agglutination and result in a negative agglutination test. It is the purpose of this study to investigate the nature of these blocking antibodies.

The sera of 5 patients with known brucellosis were separated by starch block electrophoresis. The serum fractions were studied for their brucella antibody activity. The whole serum of 4 patients with chronic brucellosis contained agglutinating antibodies as well as blocking activity. Agglutinating antibodies were demonstrated in the beta and gamma globulin fractions, whereas the blocking activity was confined to the beta globulins. The fifth patient had had a recent infection with *Br. suis*. His serum showed an extremely high agglutinin titer but no blocking activity. Electrophoretic fractionation of the serum, however, revealed the presence of blocking activity in the beta globulins. It had been hidden in the whole serum by the tremendous agglutinating activity of other fractions.

Blocking activity against brucella agglutinins was not found in the serum fractions of normal persons or of patients with unrelated chronic infections, such as tuberculosis. Combination of agglutinating fractions resulted in agglutination titers corresponding to the algebraic sum of the fractions employed. Combination of blocking fractions also resulted in an addition of blocking activity. The combination of blocking and agglutinating fractions decreased the activity of the agglutinating fractions.

Heating to 56° C. for 30 minutes or suspension in 10 per cent NaCl solution did not materially change the blocking activity of the beta fractions. Extraction with ether also failed to alter the blocking activity of beta globulin fractions. Incubation with phenol-killed *Br. melitensis*, however, completely absorbed the blocking activity of beta globulin fractions. Absorption with *Salmonella typhi* resulted in slight or no attenuation of the blocking activity.

The prozone phenomenon in the agglutination test for brucellosis is due to specific blocking antibodies. They are found in the beta globulins, whereas agglutinating antibodies are encountered in both beta and gamma globulins.

127. VARIATIONS IN A PLASMA ENZYME ACTIVITY WITH SPECIAL REFERENCE TO PATIENTS WITH THROMBOEMBOLIC TENDENCIES

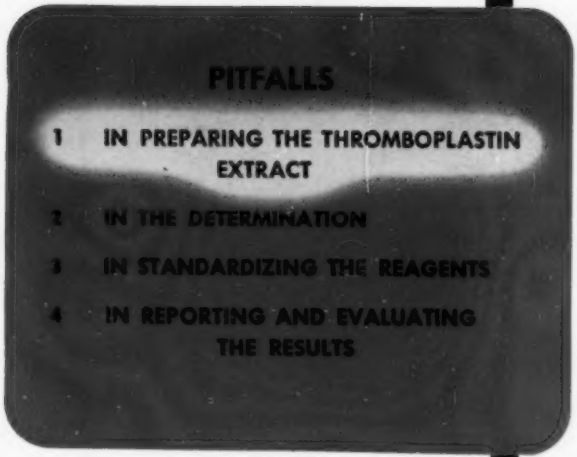
PHYLLIS M. ARSCOTT, M.A., J. L. KOPPEL, PH.D., AND JOHN H. OLWIN, M.D., CHICAGO, ILL.

(INTRODUCED BY C. BRUCE TAYLOR)

Fresh human plasma has been found capable of splitting tosyl-arginine-methyl ester (TAMe), a known substrate for thrombin, plasmin, trypsin, plasminogen activator, and the first component of complement. The level of this activity was significantly above normal in those patients having histories of past thromboembolic complications. It was high also within the first 2 days immediately following a thrombotic episode, but dropped to normal levels within the subsequent 2 weeks. High levels of activity shown by patients with long-term histories of thrombotic episodes were always reduced to normal by therapy with prothrombin-depressant anticoagulant drugs. The time required to bring about this change, however, was considerably longer than that necessary to reduce plasma prothrombin to therapeutic control levels. It should be emphasized that high levels of activity were also encountered in 2 other groups of patients which will not be discussed here, namely, pregnant women and allergic subjects.

In all cases studied the arginine esterase activity was completely inhibited by soybean trypsin inhibitor. It is not known at this time whether the activity measured is due to one or more plasma enzymes; neither is it known whether differences in enzyme activities are caused by differences in enzyme concentration, differences in inhibitor concentration, or both. Experimental data as well as theoretical considerations have led to the tentative conclusion that the activity measured is not due to the presence in plasma of free trypsin, plasmin, or thrombin. As regards a possible involvement of the first component of complement and plasminogen activator, or possibly an unknown factor, insufficient information has been obtained to support a conclusion. It is hoped that work currently in progress may lead to a more precise identification of the enzyme or enzymes being measured, as well as to a better understanding of the relationship between them and various disease states.

(We regret that Abstract 127 was inadvertently omitted from the original printed program of the Proceedings.)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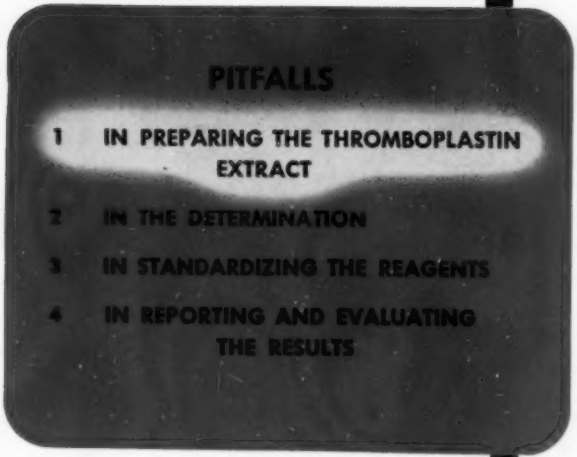
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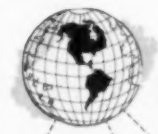

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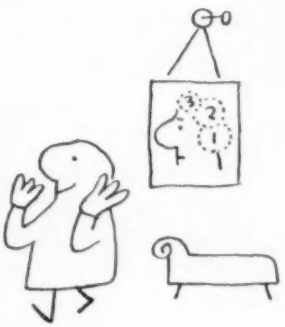

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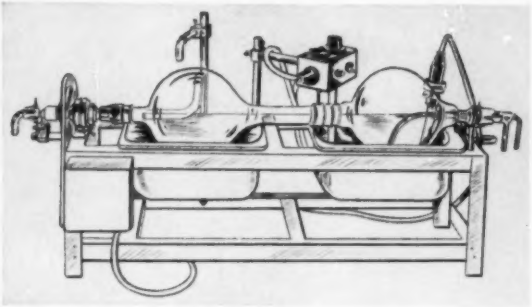

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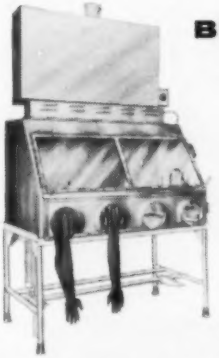

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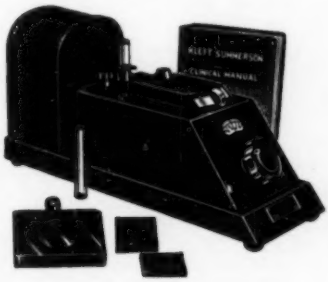

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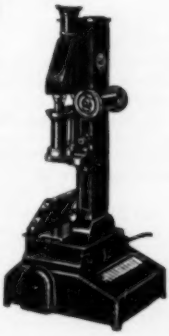

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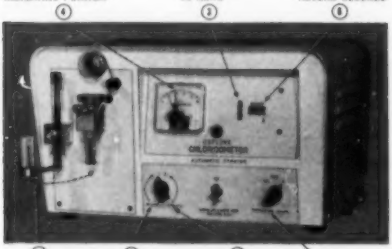

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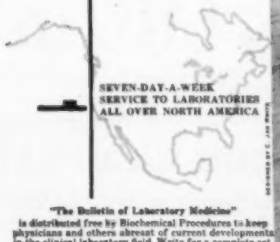

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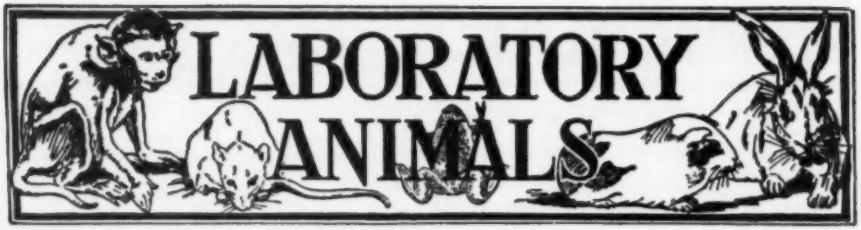

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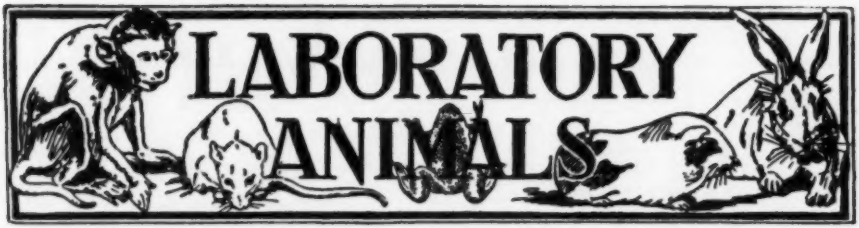

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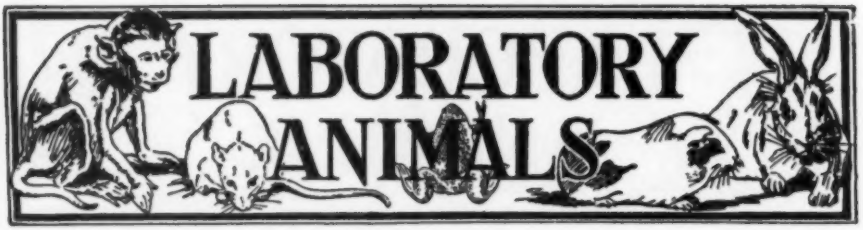

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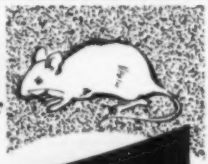

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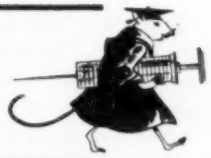

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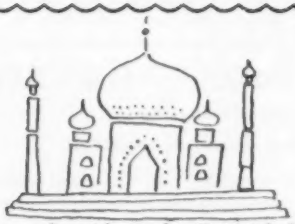

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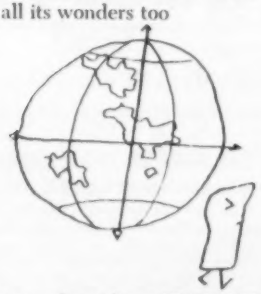

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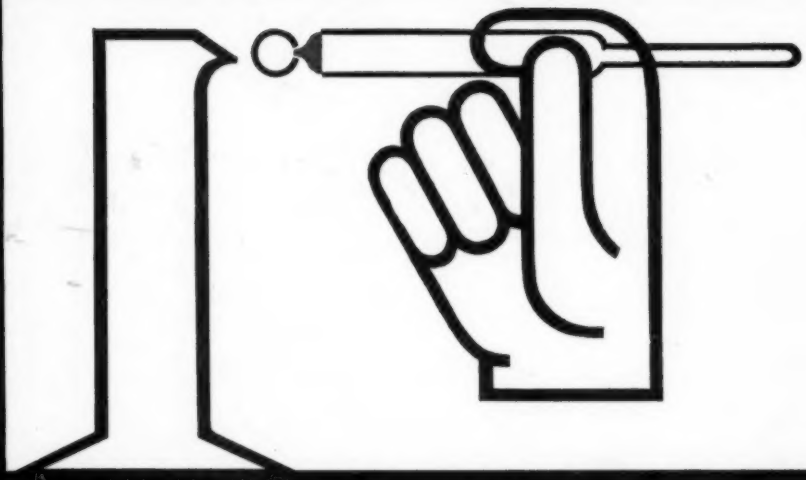

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